

1ST INTERNATIONAL CONFERENCE (IRAD-2023)

RECENT TRENDS IN IMPROVING RESISTANCE AGAINST DISEASES USING MOLECULAR GENETICS AND OMICS TOOLS

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Garden Campus, Abdul Wali Khan University Mardan



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**Recent Trends in Improving Resistance against
Diseases Using Molecular Genetics and Omics Tools
December 6-7, 2023**

**ORGANIZED BY
ABDUL WALI KHAN UNIVERSITY MARDAN,
PAKISTAN**

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VENUE OF THE CONFERENCE
Garden Campus, Abdul Wali Khan University Mardan

CHIEF ORGANISER & FOCAL PERSON

Dr. Tahir Usman



VICE CHANCELOR MESSAGE

Dear Esteemed Participants,

It is with great pleasure and enthusiasm that I extend my warmest greetings to all of you as we embark on the journey of the 1st International Conference on Recent Trends in Improving Resistance Against Diseases Using Molecular Genetics and OMICS Tools. It gives me immense pride to witness the convergence of brilliant minds from across the globe, dedicated to advancing the frontiers of science and addressing critical issues in the realms of One Health, Combatting Diseases, Antimicrobial Resistance, Livestock Improvement, and OMICS: The Future.

In the rapidly evolving landscape of scientific research, this conference stands as a testament to our commitment to fostering interdisciplinary collaboration and pushing the boundaries of knowledge. The themes of the conference are not merely topics for discussion; they represent our collective responsibility to address the challenges that threaten the health and well-being of our global community.

The concept of One Health underscores the interconnectedness of human, animal, and environmental health, emphasizing the need for holistic approaches to combat emerging diseases. In the face of ever-evolving microbial threats, the discussions on Combatting Diseases and Antimicrobial Resistance are timely and crucial. Livestock Improvement, as a theme, acknowledges the pivotal role of genetics in enhancing the productivity and resilience of our agricultural systems. Finally, OMICS: The Future encapsulates the transformative power of advanced technologies in unraveling the complexities of living systems.

As we convene to share our research findings, insights, and innovations, I encourage you to engage in meaningful discussions, foster collaborations, and explore novel avenues for research. The abstracts presented in this conference will not only contribute to the abstract book but also serve as building blocks for future breakthroughs in the field.

I extend my heartfelt appreciation to the organizing committee, keynote speakers, presenters, and participants for your dedication to the pursuit of knowledge and the betterment of our global community. May this conference be a catalyst for groundbreaking discoveries and a forum for nurturing lasting connections.

I wish you all a stimulating and enriching experience at the 1st International Conference on Recent Trends in Improving Resistance Against Diseases. Together, let us chart the course towards a healthier, more resilient future.

Best Regards,

Prof. Dr. Zahoor ul Haq (TI)

Vice Chancellor

Abdul Wali Khan University Mardan



CHIEF ORGANIZER AND FOCAL PERSON MESSAGE

Dear Participants,

It is with immense pleasure and excitement that I extend my warmest welcome to you for the upcoming 1st International Conference on Recent Trends in Improving Resistance Against Diseases Using Molecular Genetics and OMICS Tools. As the Chief Organizer and Focal Person, it is truly an honor to host this groundbreaking event at Abdul Wali Khan University Mardan in the picturesque city of Mardan, Khyber Pakhtunkhwa, Pakistan.

Our conference aims to bring together a diverse and accomplished group of researchers, scholars, and practitioners who share a common passion for advancing the frontiers of science, particularly in the crucial areas of One Health, Combatting Diseases, Antimicrobial Resistance, Livestock Improvement, and OMICS: The Future.

Over the course of two enriching days, we anticipate dynamic discussions and thought-provoking presentations that will not only deepen our understanding of the challenges we face but also inspire innovative solutions. Abdul Wali Khan University Mardan, nestled in the heart of Khyber Pakhtunkhwa, provides a fitting backdrop for this collaborative exchange of ideas.

The themes of the conference are not just topics of conversation; they represent the core issues that demand our attention and collective effort. One Health emphasizes the interconnectedness of various facets of health, underscoring the need for collaborative, interdisciplinary approaches. Combatting Diseases and addressing Antimicrobial Resistance are critical in safeguarding public health globally, and discussions on Livestock Improvement are integral to the sustainability of agricultural systems. OMICS: The Future heralds a new era of scientific exploration and discovery, powered by advanced technologies.

I sincerely thank the organizing committee, keynote speakers, and contributors for their dedication to making this conference possible and fostering collaboration. As the Chief Organizer and Focal Person, I urge participants to actively engage in sessions, network, and contribute to shaping the future of scientific research. The presented abstracts, published in the abstract book, will play a vital role in advancing global scientific discourse.

Once again, welcome to the 1st International Conference on Recent Trends in Improving Resistance Against Diseases. Let us make these two days a memorable and transformative experience for us all.

Best Regards,

Dr. Tahir Usman

Chief Organizer and Focal Person



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ONE HEALTH (OH): HUMANS, ANIMALS, PLANTS, AND ENVIRONMENT

OH-1

Plastic Pollution Exposed: Sources, Consequences, and Prevention Strategies

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ABSTRACT

Environmental pollution is considered a global issue nowadays, and immense efforts are being made to prevent it. Plastic pollution has become one of the most pressing environmental issues as plastics are accumulating worldwide on land and in oceans due to rapid population growth and urbanization. The amount of plastic garbage created has grown due to the fast population development, creating significant problems for its disposal. Plastic products are made up of various elements such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulfur. Plastics and natural materials such as rubber or cellulose are composed of very large molecules, or polymers, through a process called polymerization. Plastics are produced under specific and appropriate temperature and pressure conditions; additional plastics from industrial and municipal sources have caused headaches for agencies tasked with handling them. There are various methods for processing these materials. Various common methods to prevent plastic pollution have been investigated through this literature research. The result of the research revealed that there are two common ways to prevent the pollution of plastic products, one of which is to bury them and burn them, and the second way is the process of recycling, which can be used as a secondary product, gas, or oil. It can serve as an energy source. In conclusion, tackling plastic pollution demands a comprehensive strategy that encompasses public education and policy reforms. By encouraging sustainable practices and minimizing plastic consumption, we can join forces to create a cleaner environment for future generations.

Keywords: plastic pollution, plastic additives, plastic recycling.

Study on Incidence of Scabies in Sarkhrod District Dogs

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ABSTRACT

Scabies is an emergence infections disease which caused by mite sarcoptic scabies. This disease effect the animal and human Health and Sarcoptic mange is worldwide warranting disease. This disease commonly occurring in Dogs, Cats and goats including of Human. This disease occurring in all warm blood animals. This study was conducted in Sarkhrod district's dogs. This study started 1396/12/6 and end on 1397/1/6. The aim of this disease was to determine incidence of Scabies in dogs total cases were 30 doges examined a disease Samples were collected by skin scraping technic. After that direct method was conducted on Samples. In this study 15 doges were infected On Scabies and General Prevalence was 50% and their Significant were ($P < 0, 05$). In this study the incidence of Scabies were different ages of dog. In step one that dogs which had less from three months (2/10) were positive and their percentage was 20% in step two that doges which ages were three months to six months (5/10) sample were positive and their percentage were 50% and in that doges which had above from six months (8/10) sample were positive and percentage were 80% in the result of this study the incidence of this study were high in doges that had above from six months then other ages of doges. their significant were ($P > 0,05$) and that dog which had less than three months and that dog which had (3-6)months age the occurrence were similar by statistical analysis. Their significant were ($P > 0, 05$) so that study determined that those doges which had much age very susceptible then that doge which had less age.

Keywords: Scabies, External parasites, Dogs, Skin scraping, Sarcoptic Mange.

Identification of Immunoreactive Proteins of *S. Typhi* with Potential as Diagnostic Marker and Vaccine Candidates

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ABSTRACT

Typhoid fever, caused by *Salmonella typhi*, is a fatal disease in developing countries including Pakistan where it ranked as 6th major cause of mortality. High frequency of typhoid fever is due to the poor-hygienic lifestyle, emergence of drug-resistant strains, limited vaccine availability and poor/misdiagnosis of the disease by diagnostic tests. So, development of sensitive, specific, and rapid proteomics based diagnostics and vaccine candidates could effectively control the typhoid fever in Pakistan. The objectives of the present study was to characterize the immunoreactive proteins of clinical *S. typhi* for their application as diagnostic marker and vaccine candidates. *S. typhi* were characterized by blood culturing, PCR, 16S rRNA gene sequencing and antibiotic susceptibility profiling followed by the immunoaffinity-chromatography-based mass spectrometric approach for the characterization of *S. typhi* immunoreactive proteins. A total of 28 immunoreactive proteins were identified, 14 proteins were bounded to IgG, 4 proteins were bounded to IgM and 10 proteins bounded to both IgM and IgG. These immune reactive proteins were GlyA, HlyE, ArgI, RfbH, DapD, TufA, PflB, TrxB, GroEL, and PepD. All the identified immunoreactive proteins were highly enriched in their characteristic biological and molecular functions. The immunogenicity of selected immunoreactive proteins were also predicted *in silico* in which high immune responses were recorded, thus confirming their applicability as vaccine candidate and diagnostic marker. The present study identified several potential immunoreactive proteins which could contribute to the development of vaccines and serodiagnostic tools for the better management of typhoid fever.

Keywords: Immunoreactive Proteins, *S. typhi*, Diagnostic marker, Vaccine candidates, Pakistan

Potential of Rhizospheric Fungi to Reduce Copper and Lead Toxicity in *Triticum Aestivum* L.

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ABSTRACT

Plants have adapted to various heavy metals and environmental situations during anthropogenic evolution. The most common heavy metal contaminants are lead (Pb) and copper (Cu), which can be found in both natural and industrial settings. Microorganisms play a crucial role in the biotransformation of heavy metal-contaminated environments. To successfully remediate heavy metals, a combination of microbial strains should be used instead of a single strain culture. In this study, four rhizospheric fungus strains were extracted from *Parthenium hysterophorus* and tested for their tolerance to lead and copper. These strains were selected for their ability to promote plant growth under heavy metal stress. When inoculated to *Triticum aestivum* L. under heavy metal stress, the fungal isolates NMG, Ng, N6, and NB showed promising results in the form of increased secondary metabolites. Different concentrations of specific heavy metals (Pb25, Pb75, Cu100, Cu200, Pb25+Cu100, and Pb75+Cu200g/mL) were applied to wheat plants. The plants exposed to metal stress without microbial inoculation displayed a reduction in growth. However, when rhizospheric fungal inoculation was introduced, it not only restored plant growth and development but also enhanced the symbiotic relationship between the fungus and the host plant roots under heavy metal stress conditions. The fungal isolates were able to survive concentrations as high as Pb75+Cu200 g/mL of the selected heavy metals. The isolates have the ability to transform HMs, making them unavailable for plant uptake and accumulation, which prevents harm to plants. Apart from their bioremediation properties, some isolates can create secondary metabolites like IAA, GA, ABA, H₂O₂, Electrolytic leakage, SA, lipid, DPPH, flavonoids, phenols, and proteins, which can stimulate plant growth and make them suitable for use as bio-fertilizers and bio-remediating agents in areas with high levels of heavy metal contamination.

Keywords: Bioreduction, Bioremediation, Heavy metals uptake (lead, copper), Heavy metal tolerance, Rhizospheric fungi, *Triticum aestivum* L.

Enhancing Public Health through Modern Agronomy: Sustainable and Nutrient-Rich Practices

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ABSTRACT

This study provides a comprehensive examination of the synergy between contemporary agronomy concepts and their impact on public health through sustainable and nutrient-rich agricultural practices. We explore a range of innovative approaches, including Precision Agriculture, Sustainable Farming, Efficient Nutrient Management, Crop Diversity, Biofortification, Climate-Resilient Farming, Vertical Farming & Urban Agriculture, Digital Agriculture, and Agroecology. Each of these practices is scrutinized to uncover their intricate interconnections and profound implications for public health. Our aim is to showcase the transformative potential of modern agronomy as a dynamic catalyst for improving public health and enhancing the quality of life for individuals and communities globally. By delving into the complexities of these agronomy practices, we seek to highlight their collective contribution to advancing public health. Through the integration of cutting-edge agronomic practices, this study emphasizes the potential to revolutionize agriculture into a formidable force for enhancing public health. The multifaceted impacts extend beyond traditional agricultural boundaries, manifesting in improved food quality, heightened nutritional value, and the safeguarding of the well-being of individuals and communities. This study signifies a commitment to harnessing the power of modern agronomy to propel positive change, ultimately fostering a healthier and more energetic global society.

Keywords: Agronomy; Sustainable Agriculture; Nutrient Management; Biofortification; Public Health; Modern Farming Practices

The Pathogenic Nature of *Staphylococcus epidermidis*: A Friend and A Foe

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ABSTRACT

Staphylococcus epidermidis is the most common normal skin flora, however linked with pediatric bacteremia with significant morbidity and healthcare costs. Despite its prevalence in pediatric bacteremia, the pathogenic nature and differential markers of both host- and pathogen-associated virulence determinants are elusive. The contribution of biofilm factors in biofilm development is complex and inconsistent. Differential diagnosis and nature of infection of *S. epidermidis* is a major concern in clinical Microbiology. Hence, the study sought to evaluate the role of polysaccharide-dependent and polysaccharide-independent pathogenic determinants in biofilm development, genetic markers of discriminatory pathogenic potential and host inflammatory response in *S. epidermidis* bacteremia of pediatric origin. Through multiplex PCR and the Congo red assay, 100 isolates (n=75 clinical; n=25 community) were tested for methicillin resistance (*mecA*), formate dehydrogenase (*fdh*), and a variety of virulence factors including MSCRAMMs and the *icaADBC* operon. The isolates were typed into four groups based on the presence of specific virulent factors. The study also documented complete blood count (CBC) inflammatory markers and C-reactive protein (CRP) to understand the host-inflammatory response. The study was conducted on peads aged 0 day-7 years admitted to a Tertiary Care Centre during February 2021 to December 2021. Association of host inflammatory response was also analyzed in bacteremia caused by *S. epidermidis* with different virulent and genetic backgrounds. The *icaA* was the most significant representative module of the *icaADBC* operon that was actively expressed (85% sensitivity). *S. epidermidis* formed a robust biofilm in the presence of 5% human plasma and glucose stress. MSCRAMMs were ubiquitous and linked with biofilm positive *S. epidermidis* ($p < 0.05$). Type B clinical isolates representing methicillin resistant *S. epidermidis* (MRSE) and type C clinical isolates missing *fdh* had considerable discriminatory diagnostic potential ($p < 0.00001$). Monocytosis ($p = 0.012$) was significantly associated with *fdh* negative *S. epidermidis* bacteremia. A polysaccharide-independent mechanism has substantially replaced the polysaccharide-dependent biofilm development ($p = 0.007$). *MecA* and *fdh* are major pathogenicity and commensalism differential markers, while host inflammatory response in younger infants is adaptive in nature in *S. epidermidis* bacteremia of pediatric origin. The MSCRAMMs of *S. epidermidis* involved in paediatric bacteremia could be promising anti-biofilm therapeutic targets, markers of prompt diagnosis, and vaccine candidates.

Keywords: *S. epidermidis*, Biofilms, Pediatric Bacteremia, Methicillin-Resistant *Staphylococcus epidermidis* (MRSE), Host-Pathogen Interactions

First Seroprevalence Report of *Toxocara canis* in Different Exposure Groups in Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The zoonosis known as human toxocariasis is extremely common throughout the world, although it is mostly unreported. This investigation was carried out in the districts of Mardan, Swabi, and Nowshera in the Khyber Pakhtunkhwa province of Northwest Pakistan in order to assess *Toxocaracanis* seropositivity in various exposure sub-groups. Four hundred male individuals aged 15 and above provided blood samples; these included individuals without pets and livestock, those with livestock, those who had dogs or cats, butchers, and veterinarians or para-veterinarians. An IgG antibody to *T. canis* was detected in serum by means of a commercial ELISA kit. Each group's proportion of seropositive individuals was presented and, the chi-square or Fisher's exact test was used to assess group differences. For every subpopulation, potential risk variables obtained by distributing a questionnaire were also assessed. A significant difference was observed in the seroprevalence of individuals with no animals (5.0%; 5/100), those with dogs and/or cats in the household (8.0%; 8/100), those with livestock (18.0%; 18/100), veterinarians or para-veterinarians (24.0%; 12/50), and butchers (28.0%; 14/50) ($p < 0.001$). The overall seroprevalence of *T. canis* was 14.2%. For certain subgroups, there were notable variations in seropositivity according to economic status, educational level, and working in the fields. The results of the study show that some subpopulations in Northwest Pakistan might be more susceptible to contracting *T. canis*. Thus, the creation and application of focused preventive measures may be required.

Keywords: *Toxocara canis*, *T. canis*, Seroprevalence, Zoonoses, Risk Factors

Intestinal Worms and Associated Risk Factors with a Focus on *Taeniid cestodes* among School Children of Malakand Region, Pakistan

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ABSTRACT

The incidence of foodborne diseases, including taeniid infections, is recognized as one of the neglected tropical diseases, and rural areas of Pakistan are particularly affected. This study aimed to investigate the risk factors associated with taeniid infections among school children in the Malakand region of Pakistan. A random sampling technique was used, and the data was analyzed using GraphPad version 5. A p-value of less than 0.05 at a 95% confidence interval was considered statistically significant. Out of 360 school children, 39% (n=140) were found to have intestinal parasites, while 61.1% (n=220) did not. Taeniid tapeworm infection was present in 22.85% (n=32) of the children, with single infections in 40.6% (n=13), double infections in 28% (n=9), triple infections in 27.1% (n=8), and quadruple infections in 6.25% (n=2). Among the positive cases, 78.57% (n=110) were male and 21.42% (n=30) were female. Among the various parasites identified, *Ascaris lumbricoides* was the most prevalent, affecting 30.71% (n=43) of the children, followed by *Taenia* spp. (22.85%, n=32). Less common parasites included *Toxocara* spp., *Giardia lamblia*, and *Cryptosporidium* spp., each accounting for only 0.71% (n=1) of the cases. The study found that male students were more frequently infected than female students. Socio-economic factors also played a role: 66.66% of the children came from families with more than ten members, and children whose parents were employed had higher infection rates. Interestingly, children of illiterate parents were more likely to be infected. Regarding household conditions, 51.28% of the children lived in homes with marble floors, as opposed to concrete, cement, or earthen floors. The socio-economic factors investigated were not found to be statistically significant ($p > 0.05$) in relation to parasitic infections. In conclusion, it is recommended that health campaigns targeting taeniid infections include community outreach to address these issues and reduce the burden of intestinal parasitic infections in the region.

Keywords: parasitic infection, parasitic disease, risk factors, children, diarrhea, taenia, taeniid tapeworms, intestinal parasitic infections, *Ascaris lumbricoides*, tropical diseases

Pharmacological Studies on Diuretic Effects of Hecogenin and Hecogenin Acetate via Aldosterone Synthase Gene Modulation

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ABSTRACT

Hecogenin is a steroidal saponin found in many plant species and serves as a precursor in the manufacturing of steroidal drugs. The diuretic properties of Hecogenin and its derivative, Hecogenin Acetate remain largely unexplored, despite their potential therapeutic significance. The current study aims to investigate the diuretic activity of Hecogenin and Hecogenin Acetate in comparison with the standard diuretic drugs, furosemide and spironolactone and to elucidate its mechanism particularly focusing on aldosterone inhibition through the assessment of aldosterone synthase gene expression. Sprague-Dawley rats were subjected to various doses of Hecogenin and Hecogenin Acetate. Urine volume was measured at 1, 2, 3, 4, 5, 6, and 24 hours post-administration and urine electrolytes was measured. Additionally, animals were dosed daily for seven days, after which they were sacrificed. Blood was collected for serum electrolyte analysis, and adrenal glands were extracted for aldosterone synthase gene expression studies using conventional polymerase chain reaction (PCR). The findings indicated that Hecogenin and Hecogenin Acetate caused a significant and dose-dependent increase in urinary water and electrolyte excretion in normal rats. These effects were observed predominantly at higher doses. The results obtained were compared with the standard drugs furosemide and spironolactone. Notably, Gene expression studies of both compounds demonstrated a dose-dependent modulation of the aldosterone synthase gene in treated animals. The study establishes Hecogenin Acetate as a potent diuretic agent, surpassing the effects of Hecogenin. The observed diuretic activity is associated with aldosterone inhibition, supported by the modulation of the aldosterone synthase gene. These findings offer valuable insights into its diuretic effects and underlying molecular mechanisms.

Keywords: Diuretic activity, Hecogenin, Hecogenin Acetate, Aldosterone synthase gene, Polymerase chain reaction (PCR).

Induction of Heat Stress Tolerance in *Zea Mays L.* by using Endophytic Fungus Isolated from Selected Heat Tolerant Plants

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ABSTRACT

Maize (*Zea mays L.*) is important cash crop growing worldwide for their rich starch, proteins, fats and fiber seeds and major food source but at Global climate in the form of earth temperature, on the other hand, is a constant danger to this vital crop. The world's tropical zones are the most sensitive to rising temperatures since their plants are already stressed by the heat. As the temperature rises over its typical level, more water is lost, resulting in a physiological drought and a decline in the quality and amount of water. Fungal endophytes are used by researchers to generate biotic and abiotic tolerant plants. Present study deals with five different heat tolerant and growth-enhancing endophytic fungi. The endophytic cultures were screened for producing beneficial chemicals including primary, secondary metabolites and hormones. All plant growth promoting fungal strains were known to be helpful in alleviating heat stress by enhancing important phyto-hormones, secondary metabolites & shoot/root size, fresh/dry weight, after inoculation to maize seedlings on 25°C and 45°C. B10 2S strain results best growth-promotion and B12 2S was the least growth promoter, best IAA producer was B31S and Bc12 1L was least IAA producer, best GA producer was Bc10 1L, best ABA producer was B3 1S, best SA producer was Bc12 1L, best flavonoids producer was Bc11 2S, best Proline Producer was Bc10 1L, best protein enhancer was Bc11 2S, highest phenols producer was Bc10 1L, best lipids producer was Bc12 2S, best carbohydrates producer was Bc10 1L.

Keywords: Wheat, Endophytic Fungi, Heat stress, IAA, ABA

Detection of Gastrointestinal Helminth Parasites through Coprological Examination in Domestic Dogs in Maidan District Dir Lower, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Dogs the best friend of human being, over 400 different varieties plays role in environment in different manner. Dog's life and health are affected by different disease causing agents such as bacteria, virus and parasites. Parasites are the disease causing organisms effect the life style and health of dogs. To study the dog's intestinal parasites and their effect on human, current study was aim to find the prevalence and risk factor of gastrointestinal parasites of domestic dogs in the valley of Maidan, Dir Lower. 132 fecal samples of domestic dogs were collected and studied on both flotation and sedimentation techniques using microscopy. Overall prevalence of parasites were 59.8%. Fecal samples showed that highest prevalence were recorded for *Toxocara canis* 43.03%, *Ancylostoma Caninum* 26.58%, *Strongyloides stercoralis* 13.92%, *Mesocestoides* 18.98%, *Diphyllobothrium latum* 17.72% and *Alaria spp* 2.53%. Pups were more infected than adult while male were more than female dogs. All of the parasites have zoonotic potential. This study also shows that these parasites are easily transmissible. The results showed that there was important influx of gastrointestinal parasites among the dogs of the study area coupled with the high level of illiteracy among community members about parasites and their transmission. This shows that immediate action needs to be taken to decrease infection rates in dogs and to raise awareness in the community about zoonotic diseases and their transmission. Government may also take serious action and vaccinate the dogs and provide anti-helminthic to the owner for the treatment of the dogs.

Keywords: zoonotic disease, gastrointestinal helminth, parasites, coprological examination, dogs

Synthesis and Characterization of Ag@Fe Alloys Using *Grewia Optiva* Leaf Extract and their Bioremediation Applications

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ABSTRACT

Nanoparticles and plants play a crucial role in effectively removing heavy metals from polluted water and soil. The enhanced surface-to-volume ratio and unique chemistry of nanoparticle composites contribute to their ability to mitigate environmental pollutants. In this study, biofabricated silver iron composites (alloys) using *Grewia optiva* leaf extract was characterized for phytoremediation. Wastewater from sewage channels near Garden Campus, Abdul Wali Khan University Mardan, was collected, and various physiochemical parameters of collected waste water were measured. The hydroponic study investigated the impact of Ag@Fe alloys on *Brassica campestris* regarding plant development, photosynthetic pigments, phytochemicals and lead (Pb) accumulation. Seedlings were grown in wastewater and Pb-polluted media supplemented with 50 mg L⁻¹ of Ag@Fe alloys. Atomic Absorption Spectrophotometry assessed Pb levels in plant tissues. Pb and wastewater negatively affected seedling development, but the introduction of bimetallic nanoparticles (alloys) alleviated stress, enhancing plant growth and chlorophyll and carotenoid levels. Lead at a concentration of 50 mg L⁻¹ inhibited phytohormones, secondary metabolites, and overall plant growth. However, treatment with biofabricated bimetallic particles increased production of primary and secondary metabolites, reducing oxidative stress. While wastewater increased Pb levels, treating *B. campestris* roots and shoots with alloys reduced Pb concentrations. These findings highlighted the potential for nano-phytoremediation, demonstrating that plants can effectively remove heavy metals from contaminated water when exposed to appropriate concentrations of nanoparticles, alloys, and coreshells.

Keywords: Green synthesis, AgNPs, FeNPs, Alloys, Coreshell, characterization, Lead, *Brassica campestris*, Phytoremediation.

Morphological Identification and Acaricidal Effects of Pakistani Bee Propolis on Farm Cattle Ticks

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ABSTRACT

Propolis is produced by honey bees and used for medicinal purposes since ancient times. The Cattle ticks infest almost 80% of the world's cattle and are responsible for huge economic losses in the livestock sector. This study aims to identify the ticks infesting the farm cattle on the basis of their morphologies and to evaluate the acaricidal effects of Pakistani bee Propolis against farm cattle ticks. In the present study, two tick species were identified under the stereo-zoom microscope as *Rhipicephalus microplus* and *Hyalomma marginatum*. This was a random experiment, including 5 treatments with three replicates, and a total of 30 experimental units, 15 for each species. Each unit consists of 10 live ticks of homogeneous weight. Each tick from the experimental unit was exposed to the crude ethanolic extract of the Propolis diluted in the distilled water according to the following graded concentrations: 6.25, 12.5, 25, 50, and 100 mg/ml. The experiment was noted at different time intervals up to 48 hours. Lambda Cyhalothrin (a common synthetic acaricide) was used as positive control and distilled water as negative control. Starting from the 30-minute to 1-hour tick post-exposure, 100 mg/ml concentration has caused a higher killing rate in both tick species when compared to Lambda Cyhalothrin. A significant increase in tick mortality was recorded after 2 hours of exposure to Lambda Cyhalothrin, 50 and 100 mg/ml concentrations. After 24 hours, Lambda Cyhalothrin, 50 and 100 mg/ml concentrations caused significantly higher mortality in ticks as compared to the other concentrations. Results showed that both *Rhipicephalus microplus* and *Hyalomma marginatum* were sensitive to higher concentrations of Propolis extract. 50 and 100 mg/ml concentrations have shown very strong and comparable acaricidal effects on the cattle ticks with no significant difference from Lambda Cyhalothrin at 24 hours post-exposure time. It was found that the killing effect of Propolis extract increases with the increase in concentration and time. This illustrates the potential of Propolis extract as an alternative for tick control in cattle. This study recommends further exploration of the Pakistani bee Propolis efficacy against other pathogens and extractions of the compound inhibiting the survival of ticks.

Keywords: Ticks, *Rhipicephalus microplus*, *Hyalomma marginatum*, Propolis, prevention and control

Prevalence and Antimicrobial Resistance of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia Coli* in Slaughtered Goats in Lahore

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ABSTRACT

Antimicrobial resistance (AMR) is one of the biggest threats to global health, food security, and development today. The goal of the study was to determine the prevalence of Extended Spectrum β Lactamase producing *Escherichia coli* (ESBL *E. coli*) in goats in Lahore and to find the resistance in *E. coli* against commonly used antibiotics. A total of 250 samples were collected from May to August 2022 from Punjab Agriculture & Meat Company Slaughterhouse (PAMCO) in Lahore. A part of goat cecum was collected in Buffer Peptone Water (BPW) transported to the lab in cold conditions and were incubated at 37°C for 24 hours. The samples were inoculated onto MacConkey agar. The plates were incubated at 37 °C for 24 hours. *E. coli* was confirmed by colony morphology & various biochemical tests. The antibiotic susceptibility profile of *E. coli* isolates was determined against six antibiotics using Disc Diffusion method. Antibiotics used for the study were Augmentin, Ceftazidime, Cefotaxime, Ceftriaxone, Enrofloxacin & Oxytetracycline. ESBL *E. coli* were confirmed by Double Disc Diffusion & Double Disc Synergy tests. *E. coli* isolates (n=210) were recovered 250 samples. Of 210 *E. coli* isolates, only 09 were ESBL *E. coli* (4.28%). The highest AMR in *E. coli* isolates was observed against Oxytetracycline (95.71%) followed by Enrofloxacin (87.61%). The lowest AMR was observed against Augmentin (2.85%). *E. coli* isolates (n=172) showed resistance against three or more antibiotics indicating the problem of AMR. These findings indicate that goats slaughtered in slaughterhouse of Lahore are carrier of AMR *E. coli* including ESBL *E. coli*. These animals may be the source of transmission of AMR *E. coli* to humans through contaminated meat.

Keywords: *Escherichia coli*, *E. coli*, Antimicrobial Resistance, Extended Spectrum Beta-Lactamase (ESBL), Goats, Slaughterhouses

Enhancing Plant Immunity against *Pyrenospora tritici-repentis* (Ptr) by Targeting Brassinosteroid-Regulated Fatty Acid Biosynthesis to Inhibit ToxA Activity: A Multi-Faceted Approach

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ABSTRACT

Plant-pathogen interactions are intricate processes with profound implications for agriculture and food security. This study investigates the interplay between brassinosteroids, fatty acid biosynthesis, and the pathogen *Pyrenospora tritici-repentis* (Ptr) to enhance plant defenses by inhibiting ToxA, a known Ptr virulence factor. To achieve this, 11-day-old *Triticum aestivum* seedlings were infected with Ptr. Five days post-inoculation, we assessed growth parameters, photosynthetic efficiency, secondary metabolites, and ROS scavenging activities. As expected, Ptr infection reduced growth and photosynthesis due to lower secondary metabolite levels and subsequent ROS scavenging compared to the control. These effects were more pronounced when brassinosteroids (bL) were blocked before infection. Surprisingly, applying bL before infection improved growth and photosynthesis by increasing secondary metabolites and ROS scavenging. Further analysis of SRA profiles (SRR8816261, SRR8816277, SRR8816282, SRR8816278) from the NCBI SRA repository and GC-MS identified multiple metabolites in bL-Ptr treatment. Molecular docking and simulation using Gromacs and Mmpbsa revealed four fatty acid molecules with strong binding to ToxA, reducing its interaction with host Tsn1 proteins and diminishing its virulence.

Keywords: Brassinosteroids, Fatty Acid Biosynthesis, Ptr, ToxA, Plant Immunity, Pathogen Interactions, Computational Modeling.

Comparison of Drug Susceptibility Pattern for First Line Anti- Tuberculosis Drugs by Phenotypic and Genotypic Methods and Detection of Mutation in RpoB Gene

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ABSTRACT

Mycobacterium tuberculosis, the causative agent of tuberculosis, infects one-third of the world's population. The main obstacle to ending this infectious disease is the rapid development of drug resistance. This study aimed to find and compare the discordance between the two approaches used i.e., phenotypic and genotypic for drug-susceptibility testing (DST). Also, the mutation was analyzed in hotspot region of Rifampicin (rpoB) of these isolates. Patients suspected of tuberculosis were enrolled (n=304) and sputum samples collected at the Provincial TB Reference Laboratory (PTRL) in Hayatabad Medical Complex (HMC) Peshawar from October 2021 to November 2022. Among them 154 (50.65%) were from male and 150 (49.34%) females. MGIT 360 SIRE Kit and MTBDR plus v2 line probe assay were used for finding the DST by phenotypic and genotypic method respectively. The DynaMut server was used to determine how protein stability changes when it is mutated. The outcome of DST showed that 44 isolates (14.47%) were resistant in the presence of rifampicin, 44(14.47%) to isoniazid, 8 (2.63%) to ethambutol and 30 (9.86%) to pyrazinamide. In case of LPA, 58 (32.22%) isolates showed resistances to rifampicin and 49(27.22%) to isoniazid. The most common mutation observed was rpoMUT3 (S531L) 10%. In comparison to the MGIT 960 system, LPA is a more accurate and time-efficient method for detecting drug-resistant tuberculosis.

Keywords: *Mycobacterium tuberculosis*, DST, Rifampicin, rpoB, LPA.

Potential of *Trichoderma* Species as Biocontrol Agent against Phytopathogens of Rice

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ABSTRACT

Different ecotypes of rice (*Oryza sativa* L) have developed unique mechanisms to deal with submergence stress during course of development. Numerous research projects have been carried out to concentrate on the application of microorganisms that promote plant development to enhance plant health and productivity in different crops. *Trichoderma* spp. was acquired for this investigation from several soil samples. *Trichoderma* spp. were tested for their bio-control and antifungal properties against several plants' disease fungi. The objective of this study was to find *Trichoderma* spp. that could have an inhibitory effect on rice fungal infection. We found that *Trichoderma* spp. were effective in-vitro against rice pathogens i.e. *Fusarium graminearum*, *Curvularia lunata*, *Rhizoctonia solani*. *Trichoderma* spp. inhibited the growth of *Rhizoctonia solani* (46.1%), *Fusarium graminearum* (43.5%) and *Curvularia lunata* (33.9%) respectively in dual culture. This research can also show the significant effect of *Trichoderma* species against rice pathogens by increasing the growth of *Curvularia lunata* (74.4mm), *Fusarium graminearum* (68.8mm), and *Rhizoctonia solani* (67.8mm) respectively by cotton swab method. The in-vitro study of *Trichoderma* spp. isolate shows the positive effect on roots and shoots of pathogenic rice. *Trichoderma* spp. acts as biological control agent to inhibit the disease of rice as well as bio-fertilizer to increase the length of shoots and roots of rice plants.

Keyword: *Oryza sativa*, *Trichoderma*, *Fusarium graminearum*, *Curvularia lunata*, *Rhizoctonia solani*, rice

First Report on the Sero-Molecular Prevalence of *Brucella melitensis* in Local Small Ruminants

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ABSTARCT

Brucellosis is an important infectious and zoonotic disease that affects a wide range of animals and is caused by different species of brucella, such as *Brucella abortus*, *Brucella mellitensis*, etc. Brucellosis is diagnosed in humans and animals using a variety of serological and molecular assays, with varying degrees of effectiveness. This study aimed to investigate the sero-molecular prevalence of brucellosis in small ruminants in two districts of Khyber Pakhtunkhwa, namely Mohmand and Charsadda. For this study, 400 blood samples (n=200 each from sheep and goats) were collected in gel tubes, along with a detailed history of the animals examined. The overall prevalence of brucellosis was 13, 9.75, and 6.5% through the Rose Bengal Plate test (RBPT), *i*-ELISA, and PCR, respectively. In sheep, the incidence rate of brucellosis was 13.5 and 7% through RBPT and *i*-ELISA while PCR confirmed 5% *B. abortus* and 2% *B. melitensis*. In goat, the incidence rate of brucellosis was 12.5 and 12.5% while PCR confirmed 2% *B. abortus* and 4% *B. melitensis*. These results suggested that *B. melitensis* was found more prevalent in goats as compared to sheep whereas *B. abortus* was detected in higher numbers of sheep compared to goats through PCR. PCR is a more sensitive, efficacious, and time-saving test for the diagnosis of brucellosis.

Keywords: Brucellosis, small ruminants, serological, PCR, Risk factors, Charsadda, Mohmand

Characterization of Quinolones Resistant *Clostridium perfringens* Toxinotype D

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ABSTRACT

Clostridium perfringens toxins play role in causing pulpy kidney disease poisoning as well enterotoxaemia. To combat antimicrobial resistance: curbing use, regulations and execution to antimicrobial usage in food along with withdrawal period is necessary. Aim of study was to optimize the toxins production by indigenously characterized *C. perfringens* type D isolates (n=03) under various physicochemical parameters, a lead towards local vaccine production in Pakistan. Indigenous isolates were characterized on the basis of 16S rRNA and MW349974.1, MW341428.1, MW332258.1 accession numbers were received from NCBI GenBank. Isolates were identified as toxinotype D through PCR toxinotyping. Quinolones antibiotic susceptibility testing revealed that isolates observed susceptible to enrofloxacin and resistant to ciprofloxacin and ofloxacin. Optimization of toxins production was determined under the influence of physical and chemical parameters. Alpha and epsilon toxin production in reinforced clostridial medium (RCM) broth was observed higher at 37°C after 24h incubation by MW332258.1. Under the influence of 0.2% glucose and 0.3% tween 80 supplementation in RCM, greater production of alpha and epsilon toxin units was observed by MW332258.1. Under optimized physicochemical parameters, maximum toxins units were observed; MW332258.1 isolate is excellent candidate could be used to produce maximum toxin units for vaccine production at industrial scale.

Keywords: Antibiotic resistance, *clostridium perfringens* toxinotype D, glucose, hemolytic units, pulpy kidney disease.

Use of Critically Important Antimicrobials (Cias) in Poultry Health Clinics, Nangarhar Province Eastern Afghanistan

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ABSTRACT

The international one health related organizations (WHO, OIE and FAO) are developing new guidelines and policies for the safety of antimicrobials (AMs) and their use particularly Critical Important AMs (CIAs) of medically and veterinary importance globally based on related data about the use and susceptibility of AMs. The data related to the use AMs is scanty related to Afghanistan in poultry sector where use of AMs is common drug used for health protection and growth support in poultry sector. This study was designed to determine the use of AMs specially CIAs in Poultry Health Sector at Nangarhar province Eastern Afghanistan. The data is collected through questioner and interview from ten poultry clinics and retailers drug stores selected randomly and the data was compared with WHO and OIE lists under CIAs of veterinary, human concern. The results of study revealed that the total quantity of AMs used in treatment and prophylaxis was 25.482 tons, for one year which consist the percentages of different AMs classes: Tetracyclines 22.27% (doxycycline 18.00 and oxytetracycline 4.27), Aminoglycosides 16.36% (neomycin), Macrolides 12.275% (Tylosine, 7.55 and tilmycosin 4.72) fluoroquinolones 11.456% (Enrofloxacin 6.20 Ciprofloxacin, 4.63 and norfloxacin 0.61) Aminopenicillins 10.367% (Amoxicillin), Polymyxins 8.075% (colistin), Amphencoles 7.72% (florophenicol) Sulphonamides 7.18% (Sulphadiazine 2.99, Sulphapyridazine, 3.35 Sulphacloropyridazine 0.68), Diaminopyridines 1.29 (trimethoprim), Quinolone 0.477% plorotiamules 0.42 % (tiamulin) antivirals 0.23% (Amantadine), lincosamides 0.99% (lincomycin) and Aminocyclitoles 0.88 (Spectinomycin) respectively. The CIAs of medical importance make 58.5% where highest priority 31.81% (tylosin 7.55, tilmycosin 4.70, Colistin 8.075, ciprofloxacin 4.53, enrofloxacin 6.20 and norfloxacin 0.61) and high priority 26.697% (Neomycin 16.36 and Amoxicillin 10.336) make total antimicrobials used by 78 poultry clinics. In conclusion, the high amount usage of AMs in treatment and prophylaxis of disease particularly CIAs of medical important can case a severe problem of public health concern and it is required to develop policies for the reduction of AMs particularly CIAs.

Keywords: Antimicrobials, CIAs, Rational use, poultry Clinics, Active ingredient

Detection of Bio-Active Compounds in *Aesculus indica* Wall. Ex. Camb. and their Anti Bacterial Activity Against Selected Human Pathogens

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ABSTRACT

Traditional medicine makes considerable use of medicinal plants to treat a variety of infectious diseases in people. Finding new antimicrobial compounds with minimal side effects is the most crucial phase in microbiological research because resistant organisms to antibiotics is one of the biggest problems in the medical field. Using a Fourier Transform Infrared Spectrometer (FTIR) and a Gas Chromatography-Mass Spectrometer (GC-MS), the current study aimed to investigate the antibacterial activity of various organic extracts of *Aesculus indica* fruit and leaves as well as the identification of key functional groups and phytoconstituents. The extracts were tested for antibacterial efficacy against four multidrug-resistant (MDR) strains: *Shigella dysenteriae* (ATCC11835), *Salmonella typhi* (ATCC14028), *Escherichia coli* (ATCC9739) and *Pseudomonas aeruginosa* (ATCC9027). The study revealed that the extracts possessed antibacterial activity in a dose dependent manner. Among the selected bacteria, maximum growth inhibition was observed for *Salmonella typhi* with 18 mm, zone of inhibition at 100µl concentration in methanolic extract of fruit, *Pseudomonas aeruginosa* showed maximum growth inhibition of 19.5mm at 100µl in methanolic extract of fruit, *Escherichia coli* maximum growth inhibition observed with 17.7mm at 100µl in ethanolic fruit extract, while the *Shigella dysenteriae* showed a zone of inhibition of 17.3mm at 100µl in ethanolic leaves. The phytochemical screening of both the extracts revealed the presence of flavonoids, saponins, tannins and steroids. FTIR analysis confirmed the presence of various functional groups of secondary metabolites such as Alkyl halides, Carboxylic acid, Polysaccharides, aromatic group, alkyne, alcohols, nitro compounds, alkenes, alkyl group, Alkene, Polyethylene's and phenol. The GC-MS investigation led to the identification of diverse phytoconstituents in the extract. The data illustrated that 57 compounds are present in the extract. The main constituents in the compound are *Oleic Acid*, *Octadecanoic acid*, *n-Hexadecanoic acid*, *Neodecanoic acid*, *Trimethylsilyl 20-acetoxy-3,6,9,12,15,18-hexaacosan-1-oate*, *Dihydroxyacetone*, *1,3-Dihydroxyacetone dimer* and *Hydroxy-Acetaldehyde* which are dominantly present in ethanolic extract of fruit. The findings of the current study have opened the way for the development of herbal medicines for a number of microbial illnesses that are life-threatening employing *Aesculus indica*, which could result in the creation of new therapies for microbial infections that are resistant to current antibiotics.

Keywords: Antibacterial activity, FTIR, GC-MS, *Aesculus indica*, Medicinal plant, Phytochemicals

Morphological and Molecular Identification of Cystic Echinococcosis in Livestock Population of District Mardan

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ABSTARCT

The smallest canine cestode, belongs to the genus Echinococcus, is the main cause of zoonotic disease known as hydatidosis or cystic echinococcosis (CE). This study investigated the morphological and molecular identification of CE in livestock of District Mardan. A total of 542 animals including buffaloes (n=216), cattle (n=138), sheep (n=82), and goats (n=106) were examined for echinococcosis during March – June 2022 at different slaughter-houses/abattoir in district Mardan. These areas were visited for the collection of Cysts from the infected livestock population. The data was recorded through questionnaire. For the morphological confirmation, the fluid of each collected cyst was examined through microscope. Furthermore, specific (Nad1 gene) targeted in the extracted DNA generated an Amplicon of 200bp by PCR. The overall prevalence of CE was 37.5% (203/542). Microscopically identified rate of fertile hydatid cyst in the positive samples was 157/203 and the rate of sterile hydatid cyst in the positive samples was 46/203. Furthermore, the hydatid cysts were confirmed in 54.7% out of 203 samples by PCR. Among 108 samples of buffaloes, 63(58.3%) and 58 samples of cattle, 27(46.6%), while among 23 samples of sheep, 12(52.2%) and 14 samples of goat, 9(64.7%) were detected by PCR for the cystic Echinococcus species. The prevalence were determined in different species of livestock animals which include buffaloes 50% (108/216 is the total number of buffaloes), cattle 42.02% (58/138 is the total number of cattle), sheep 28.04% (23/82 is the total number of sheep), and goats 13.2% (14/106). The infection was relatively more prevalent among females 43.9% (139/316) Buffaloes (73/129), cow (45/93), ewe (16/49) and doe (5/45) than males 28.3% (64/226) Buffaloes (35/87), bulls (13/45), ram (7/33) and bucks (9/61) and animals of older age greater than 5 years. CE cases were more found in rural area 161/314 (51.3%) than urban area 42/228 (18.4%). In this study the overall prevalence of Cystic echinococcosis in livestock population was identified in the lungs 127/542 (23.43%) and the livers are 75/542 (13.83%) so the lungs are more infected than livers. The results of this study can serve as a basis for future research on the epidemiology and control of cystic echinococcosis in the livestock population of Mardan and other similar regions. It is necessary to promote awareness and implement control measures to prevent the spread of this zoonotic disease.

Keywords: Cystic echinococcosis, hydatid cyst, zoonotic, Microscopy, PCR

Isolation of Antibiotic Producing Bacteria from the Soil of Wheat Field Mardan

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ABSTRACT

Antibiotics are the most used secondary metabolites, produced by a variety of soil microorganisms such as bacteria and fungus and used in a wide range of applications. The majority of antibiotics used today are derived from bacteria. Although soil is naturally rich in bacteria capable of producing antibiotics, the frequency with which this occurs at ecologically important levels has remained unclear. However, over the last decade, genetic and molecular approaches have been used to show conclusively that microorganisms in the rhizosphere manufacture a variety of antibiotics, even under field conditions. Soil sample from wheat field Mardan was screened for antibiotic producing microorganism. Three bacterial strains have been identified. Cellular features, colony morphology, and biochemical tests were used to identify the bacteria. The emergence of the antibiotic resistance and need of broad-spectrum antibiotics is in focus and demand. Soil samples were collected from wheat field Mardan and analyzed for antibiotic production. After primary screening bacterial isolates were identified as bacillus and streptococcus through biochemical characterization and microscopic identification and checked for antibiotic activity against some common gram negative and gram-positive bacteria namely *staphylococcus aureus*, *E. coli*, *klebsiella* and *pseudomonas* using agar well and disc diffusion method to investigate antibiotic producing microorganism. The antibiotic test indicate that *bacillus* and *streptococcus* showed antimicrobial activity and produces zones of inhibition. *Bacillus* and *streptococcus* have the potential to produce antibiotics and can be used to control microbial growth in future.

Keywords: Antibiotics, soil, microorganisms, wheat, Mardan.

Analysis of Exon 27 of Atp-Binding Cassette C1 Transporter for Genetic Variations in Type 2 Diabetic Patients from Mardan, Pakistan

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ABSTRACT

ABCC1 is known as multidrug resistance-associated protein 1 (MRP1), and it is a member of the ABCC subfamily. ABCC1 is a 190 KDa transporter protein which is located on chromosome number 16p13.1. It is comprised of 31 Exons encoding a protein of 1531 amino acids. In humans, MRP1/ABCC1 is approximately present in all tissues that are important like kidney, lungs, testes, spleen, thyroid gland, urinary bladder, adrenal gland, placenta and central nervous system. In diabetic and hyperglycemic patients, ABCC1 is potentially important for the regulation of reactive oxygen species (ROS) production in vascular cells. The objective of the existing study was to find genetic variations in Exon 27 of ABCC1 gene responsible for type 2 diabetes mellitus in the population of Mardan. 20 diabetic blood samples were collected in which 4 samples were from normal individuals and 16 samples were collected from diabetic patients. DNA was extracted from all samples with the help of the phenol chloroform method. Exon 27 of ABCC1 gene was amplified through polymerase chain reaction by using a special set of primers. The amplified products were then processed for vertical gel. In future the PCR products can further be used for sequencing of exon 27 to find genetic variations in type 2 diabetic population

Keywords: ABCC1, Exon 27, PCR, Type 2 Diabetes, Mardan.

Comparison and Prevalence of *Vulvovaginal Candidiasis (Candida vaginitis)* Among Day Scholars and Hostelite Students of Shaheed Benazir Bhutto Women University Peshawar

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ABSTRACT

A yeast (or type of fungus) called *Candida* is the infection that results in candidiasis. In a human body where we have mucus such as mouth, throat, gut, and vagina most of time *Candida* does exist without being infectious. If circumstances inside the vagina change to favour *Candida*'s growth, an infection may result. Infections can become more likely due to factors including hormones, medications, or immune system changes. Vaginal yeast infection is the common name for candidiasis in the vagina. Various other names for this ailment include *Candidal vaginitis*, vulvovaginal candidiasis, and vaginal candidiasis. There is no such thing as a sexually transmitted infection for a vaginal yeast infection. The Shaheed Benazir Bhutto Women's University in Peshawar selected female students from several departments in hostelite and day scholar to participate in the current study. 130 female students in total participated in the study. According to this study, vulvovaginal candidiasis (VVC) affects 30 to 50 percent of women at some point in their lives. Pruritus or a burning sensation (27%) and dysuria (33%) are common symptoms. VVC is typically brought on by *Candida albicans*, *Candida glabrata*, or *Candida krusei*. The main lifestyle variables that increase the chance of developing VVC include frequent sex, using contraception, and vaginal douching. Students should get recommendations for treatment, education and prevention of vaginal candidiasis through appropriate hygiene practice, regular exercise, and natural remedies, especially in the genital area, to avoid fungal infection. Clotrimazole (72%), fluconazole (14%), and nystatin (6%), were the three medications that were prescribed the most frequently.

Keywords: *Candidiasis vulvovaginal*, *Candida albicans*, antifungal agents, risk factors, hygiene practices

Effect of Carbon Nanotubes on Seed Germination, Callus and Shoot Formation and Secondary Metabolites Production in *Moringa oliefera*

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ABSTRACT

This research work was designed to study the effects of carbon nanotubes (CNTs) on seeds germination, callus and shoot formation and secondary metabolites production in *Moringa oliefera* callus and shoot culture. *Moringa* seeds were surface sterilized with ethanol (70%), sodium hypochlorite (2.5%) and sterilized distilled water. For sterilization, seeds were removed from pods with the help of scalpel and blades and surface sterilized by washing several times with tap water to remove dust and surface contaminants. Seed coats were removed from the sterilized seeds in aseptic condition. Seeds were cultured on MS basal medium containing 3% sucrose, 0.9% agar, 5.7 pH, and different concentrations of CNTs (0.5, 1, 2, 3, 4 and 5 mg/l) and incubated at 25 ± 1 °C for germination. Similarly, the seeds were cut into 2 or 4 pieces and then cultured on the same medium with addition of BAP (6-benzylaminopurine) (0.5, 1.5, 2.0 mg/l) and NAA (1-Naphthalene acetic acid) (0.5, 1.5, 2 mg/l). Earlier germination was recorded on MS medium containing CNTs (2-5 mg/l). Full germination was accomplished within 15 days of culturing the seeds. Highest callus formation was achieved on MS medium containing 2.0 mg/l BAP and NAA with 4.0 mg/l CNTs. The research is still going on and we expect further results to be achieved. The preliminary results show that CNTs can have significant effect on *Moringa* seeds germination, callus and shoot formation. We also expect a significant effect on secondary metabolites production in callus or shoot culture of *Moringa*.

Keywords: *Moringa oliefera*, multi-walled carbon nanotubes, morphological characteristics, secondary metabolites

Exploring the Biological Application of Exopolysaccharides Derived From Endophytic Fungi

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ABSTRACT

Endophytic fungi residing inside the plants have emerged as intriguing source of bioactive secondary metabolites with diverse biological applications. Among these compounds, exopolysaccharides (EPS) have gained significant attention for its potential therapeutic role in various human ailments. The current study aimed to isolate exopolysaccharides from endophytic fungi of wheat and explore their biological applications using different biological assays. Endophytic fungal strains showing high EPS content isolated from wheat was inoculated in the broth medium containing yeast extract, peptone, and dextrose at 25°C for 32 hr on a shaking incubator. The EPS was precipitated from the broth culture with cold ethanol and further purified through gel filtration chromatography. The biochemical characterization of the obtained EPS confirms the presence of total carbohydrate contents while uronic acid, unsaturated uronic acid, pentose and hexose were determined as their monosaccharide units. The biological potential of the EPS isolated from endophytic fungi of wheat were determined using various biological assays including DPPH assay, α -amylase assay, α -glucosidase assay and antibacterial assay, etc. Wheat endophytic EPS exhibited strong antioxidant potential by scavenging the DPPH free radical with an effective EC₅₀ value of $69.17 \pm 0.98 \mu\text{g/mL}$ while highest concentration that is at $200 \mu\text{g/mL}$ its effectively scavenged the DPPH free radical by $89.34 \pm 1.09\%$. EPS obtained from wheat endophytes significantly ($P=0.05$) inhibited the α -amylase activity (IC₅₀ was $193.48 \pm 1.67 \mu\text{g/mL}$) and α -glucosidase (IC₅₀ was $217.72 \pm 3.47 \mu\text{g/mL}$). The results showed that fraction EPS at $100 \mu\text{g/mL}$ was able to induce $7.31 \pm 0.96 \text{ mm}$ and $8.18 \pm 1.23 \text{ mm}$ zone of inhibition against *S. aureus* and *S. typhi*. Our study concludes that by exploring the therapeutic potential of EPS, the designed research work not only contributed to our understanding of endophytic fungi but also paved the way for the development of innovative approaches to enhance human health and well-being. In a world seeking sustainable, eco-friendly and natural solutions, exopolysaccharides extracted from endophytic fungi represents a promising frontier in biomedicine research.

Keyword: exopolysaccharides, endophytes, fungi, antioxidant, wheat

Hepatoprotective Effect of Cress Seeds Carbohydrates against Chromium-Induced Liver Toxicity in Mice

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ABSTRACT

Heavy metals represents an important problem due to its toxic effect and accumulation throughout the food chain which leads to serious ecological and health hazards as a result of their solubility and mobility. Polysaccharides are complex carbohydrates made up of repeating units of monosaccharaides and have been explored as biosorbents for heavy metals and other pollutants due to their high availability, low cost, and biodegradability. The aim of the current study was to extract and partially purify carbohydrates via size-exclusion chromatography (SEC) from seed coat mucilage of garden cress and to investigate its hepatoprotective against oxidative liver damage induced by hexavalent chromium (Cr (IV)). The results revealed the presence of pentose, hexose, saturated and unsaturated uronic acid contents. Among all the screened samples for their antioxidant potential through DPPH assay, significant EC₅₀ value was shown by fraction DF73 as its EC₅₀ was 125.26 ± 1.88 µg/mL, followed by fraction DF48 and DF62 having EC₅₀ value of 148.29 ± 2.67 µg/mL and 220.07 ± 6.52 µg/mL, respectively while that of ascorbic acid was 25.63 ± 4.32 µg/mL. *In vitro* lipid peroxidation assay was performed on mice tissue samples where at 250 µg/mL, DF73 inhibited the lipid peroxide production by 55.15% which was increased to 77.02% at 500 µg/mL and 90.99% at 1000 µg/mL. *In vivo* assays showed that the administration of CSC, and its fraction including DF73, DF62 and DF48 has potential hepatoprotective effects against Cr-induced hepatotoxicity, possibly brought about by improving liver function, increasing antioxidant status, and reducing lipid peroxidation. Furthermore, the liver histology of normal and chromium-IV stressed mice providessupportive evidence for the hepatoprotective nature of cress seed mucilage carbohydrates. These results may provide supportive evidence for the exploitation of cress seed carbohydrates as hepatoprotective agent against oxidative liver damage induced by hexavalent chromium.

Keywords: hepatotoxicity, heavy metal, chromium, oxidative stress, carbohydrates

Comparative Performance of Different Peach Varieties Budded on Swat Local Peach Root Stock

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ABSTRACT

The present study entitled as “Comparative performance of different peach varieties, budded on swat local peach root stock” was performed in the field of Horticulture Research Institute (HRI) at National Agricultural Research Institute (NARC) Islamabad during year 2023. The aim of the study was to determine the growth performance of different Peach varieties (scion) budded on Swat Local Peach Root-stock. During the study, we used 10 different Varieties of *Prunus persica*, i.e Early Grand, Spring Crest, Spring Belle, Coronet, Early Maria Delizia, NJC-84, Maria Bianca, Golden, Late Maria Bianca, and Indian Blood is scion varieties which were budded on swat local peach root-stock. These varieties were budded on three-year-old Swat local peach root-stock. The experimental results showed that Scion varieties depicted variant but significant effect on all morphological parameters studied during the trial. For instant, highest green bud percentage (97.67%) was observed for Coronet variety while Maria Bianca var. exhibited the lowest (50%) green bud rate. For sprouted bud percentage, early Maria Delizia var. displayed the maximum rate (97%), whereas Late Maria Delizia exhibited the minimum rate (48.27%). Similarly, Stem length measurements indicated that the variety Early Grand attained the greatest length (23.33 cm), in contrast to NJC-84, which displayed the shortest (7.22 cm). The assessment of shoot diameter revealed Early Grand with the max. diameter (4.11 mm) and Golden with the min. stem diameter (2.03 mm). The number of leaves per plant was also analyzed, with Spring Belle var. registering the highest count (23.22), and NJC-84 recording the lowest (11.78). These findings showed that different peach varieties have variant degrees of significant influence on various growth parameters when budded on same (swat local) peach root-stock.

Keywords: budding, growth parameters, peach root stock, prunus persica, rootstock, morphological variation, horticultural performance

Role of Growth-Promoting Fungal Endophytes for Induction of Steviol Glycosides in Hydroponically Grown *Stevia rebaudiana*

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ABSTRACT

Stevia rebaudiana is a zero-calorie natural sweetener plant, and the sweet taste of stevia comes from heat-stable metabolites called steviol glycosides, including Stevioside, Steviolbioside, Rebaudioside, and Isosteviol. This study explores how growth-promoting fungal endophytes influence the induction of steviol glycosides (SGs) in *Stevia* plants grown in a hydroponic system. Inoculating *Stevia* plants with fungi in a hydroponic system enhanced plant growth promotion, measured through increased levels of primary metabolites (carbohydrates, proteins, and lipids) and secondary metabolites (IAA, salicylic acid, phenols, flavonoids, sugar, proline, and tannins). The MAJ-3 and MAJ-5 fungus-treated plants were selected to measure the levels of stevioside and rebaudioside-A using (HPLC) and GC-MS analysis. Furthermore, the transcript level of fifteen important genes involved in the SGs biosynthesis pathway was measured by qRT-PCR. The study illustrates the positive impact of endophytic fungi in hydroponic systems on *Stevia* plants, leading to the increased production of a high quantity of Steviol glycosides for industrial purposes. Additionally, the study revealed that the culture filtrate has the potential to enhance plant growth at the molecular level. Both primary and secondary metabolites were augmented, and the genes involved in the Steviol pathway expression showed increased compared to the control plants. The data analysis of qPCR revealed that all genes, except *SrGGDPS*, were enhanced compared to the control. However, more study is needed to analyze the molecular and genetic basis of these three endophytic strains in stimulating *Stevia* plant growth under a hydroponic system.

Keywords: *Stevia rebaudiana*, Steviol glycosides, Hydroponic system, HPLC, GC-MS.

Frequency Distribution of ABO Blood Groups and Rh factor in the Human Population of Town Yarhussain District Swabi Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The ABO blood group system was the first human blood group system to be discovered by Landsteiner in 1900. The second type of blood group is the rhesus system. Besides playing an integral role in blood transfusion service, blood groups are also useful in inquiring about populace hereditary qualities, migration patterns, settling medico legitimate issues. The present study of blood group and Rh factor distribution was studied during March, 2020 to July, 2020 in Yarhussain town of district Swabi in different age groups of both males and females to understand ABO and Rh distribution in human population of this area and provide updated ABO and Rh population frequency distribution. Three hundred twenty (320) blood samples of both males and females were randomly selected for ABO and RH blood groups for this study. The blood of each person was collected on glass slide through finger prick and blood grouping were performed using commercially available antisera. Blood group B was the highest with the percentage frequency of (38%), followed by group A (24%), O (20%) and the least frequent was AB which is (16%). The Rh-D distribution also varies among the four blood groups. The total percentage of Rh-D positive was 79% and Rh-D negative was found to be 21%. The blood group is important as it's save lives when transfusion is needed. This study will helpful for medical diagnosis and multipurpose future utilities like supplying blood to needy patients during emergency. The current and future tendencies of blood groups and Rh-D factors distributions in Yarhussain town of District Swabi.

Keywords: blood groups, Rh factor, distribution, Swabi

Endophytic Fungal Isolates Enhances the Physicochemical Characteristics and Mineral Profile of *Zea Mays L.* under Salt Stress

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ABSTRACT

Maize (*Zea mays* L.) is an important crop but its production can be severely limited by salt stress. Salinity and its associated land-damaging impacts are serious environmental issues for agricultural productivity, affecting approximately 800 million hectares of land globally. Aside from soil salinity, irrigation with salty water has been identified as a critical yield-limiting factor for increasing agricultural yields and productivity, particularly in low-lying coastal regions of various countries. The present research was rationalized to investigate the biophysical and biochemical aspect of maize plants and to exploit the endophytic fungi for the growth promotion of maize under intense salinity stress. MA2 and MA5 were isolated from the seeds of *Moringa oleifera* L., which ably produced a sufficient amount of IAA, proline, phenols, and flavonoids. The symbiotic association of MA2 and MA5 promoted the growth response of maize compared with the non-inoculated plants under salt stress. According to current findings, salt stress (200mM) had adverse effects on photosynthetic pigments concentration and endogenous IAA with increase in total lipids, total protein, total sugar total phenolic contents. Maize plants espouse to salt stress enhanced proline content, lipid peroxidation and anti-oxidant activity. However, MA2 AND MA5 inoculation, reversed the adverse effects of the abiotic stress by restoring chlorophyll a, chlorophyll b concentration, total lipids, protein, sugar, phenolic contents, Flavonoids, antioxidant enzyme activity (catalase, ascorbate peroxidase), and proline content to optimal levels adequate for maize plant development enhancement and salt stress reduction. Additionally, the inoculated maize plants under salt stress condition lower the Na⁺, Cl⁻ and elevate the N, P, Ca²⁺, K⁺, and Mg²⁺ content than non-inoculated plants. The current study supported the use MA2 and MA5 isolates for establishing the bipartite mutualistic symbiosis, increased salt resistance by modifying physiochemical properties and root-to-shoot ion and mineral element translocation, rebalancing the Na⁺/K⁺, Na⁺/Ca²⁺ ratio of maize plants under salt stress and used as biofertilizer in saline condition.

Keywords: endophytic fungi, proline, salinity, maize, *Zea Mays*, endogenous hormones, IAA, Ions

Association of Body Mass Index with Diabetes Mellitus Blood Pressure and Breakfast among the Students of Abdul Wali Khan University Mardan

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ABSTRACT

BMI have been related with various disease phenotypes, like type - 2 diabetes mellitus, Blood pressure, Malaria, Cardiovascular and also related with Breakfast. The major risk factor for DM and BP is an elevated BMI, furthermore other potential risk factors are unhealthy diet, genetic and environmental factors etc. Monitoring all the known factors which leads to an increased BMI can be a better therapeutic option. Therefore, this study was carried out to investigate the significance of increased BMI among the young population of Mardan including AWKUM. In this study the associations of BMI with breakfast consumption were investigated for the first time in district Mardan of Khyber Pakhtunkhwa (KP) Pakistan. All the experiments determining the blood glucose levels and blood pressure checkup were carried out in Biochemistry Laboratory, AWKUM. The blood pressure of each student was measured through digital Sphygmomanometer. When the measurement was completed, readings of systolic and diastolic blood pressure and the pulse rate were displayed on the digital panel. For glucose measurement the blood sample were taken by pricking finger with help of lancet and took the reading by using glucometer. A total of 564 volunteer participants comprising (male gender, n (%) = 111(20) female gender = n (%) 453(80) were enrolled in this study. The result indicated an increased BMI > 30 among the young population (age range: 18-25 years, n = 564) of the university. Observed risk factors were lack of awareness about BMI, lack of exercise, heavy consumption of unhealthy food including mainly junk food and carbonated drinks, unhealthy habits related to breakfast consumption etc.

Keywords: Body Mass Index, BMI, obesity, Type 2 Diabetes Mellitus, hypertension, dietary habits

Exploring Biological Activities and Exopolysaccharides from Endosymbiotic Fungi

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ABSTRACT

Endophytic fungi, residing in various plant parts, play a pivotal role in enhancing plant biodiversity. Through the secretion of a myriad of biochemical compounds, these fungi contribute significantly to the plant's defense mechanisms against pathogens. The symbiotic interaction activates distinct pathways that fortify the plant's resilience, with the production of antibiotics being a noteworthy aspect. This research focuses on the comprehensive evaluation of biological activities and polysaccharides derived from endosymbiotic fungi, specifically exploring their symbiotic relationship with plants. The secondary metabolites released by endophytic fungi exhibit toxicity against various insects, ensuring the overall health of the host plant. Additionally, these fungi influence ion movement within plant cells, crucially controlling plant physiology. Notably, endophytic fungi assist in nutrient uptake, fixing nitrogen and phosphorus, thereby promoting essential macro and micronutrient acquisition for optimal plant growth. Moreover, the research sheds light on the significant role of exopolysaccharides secreted by endophytic fungi. These compounds exhibit diverse applications, ranging from anti-tumor properties to their potential in various industries, such as food production and the development of biodegradable plastics. In particular, the exopolysaccharides' effectiveness in tumor treatment and their antioxidant, immunostimulatory, antibacterial, and anti-inflammatory activities are explored. The study emphasizes the efficient production of exopolysaccharides by endophytic fungi in marine environments, highlighting their role in maintaining ecosystem balance. Notably, the anti-diabetic activity of these exopolysaccharides adds to their significance in the realm of medicinal applications. The research concludes by underlining the broader implications of endophytic fungi and their exopolysaccharides, providing a foundation for further exploration in areas such as pharmaceuticals, biotechnology, and environmental conservation.

Keywords: Endophytic fungi, exopolysaccharides, anti-bacterial, anti-inflammatory.

Investigation the Effect *Calotropis procera* Supplementation of Bone Health, Meat Quality, and Muscle Traits in *Oryctolagus cuniculus* Rabbits

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ABSTRACT

The current study aims to determine the effect of locally occurring plant, *Calotropis procera* leaves powder (CPLP) as feed additives for bone health, meat quality, and muscle characteristics of New Zealand White rabbits (*Oryctolagus cuniculus*). A total of 64 Rabbits (56 days old) were randomly divided into four groups having four replicates (n=4) in group each (4 groups × 4 replicates × 4 rabbits). A control group received a standard diet, whereas the other three groups received standard diet supplemented with 0.4%, 0.6%, and 0.8% CPLP. The plant powders were mixed with the commercial rabbit pellet diet to provide a dose of 4g, 6g, and 8g per kg of feed. Body weights and feed intake were properly recorded on a weekly basis. The experiment was conducted in a completely randomized design in a suitable environment. On day 42, two rabbits per replicate were euthanized to collect samples of breast muscle and tibia bone. The CPLP supplementation significantly ($P \leq 0.05$) decreased the pH of breast muscle and ash percentage of tibia bone. The diameter of breast muscle fibers and weight and weight length index of tibia bone significantly ($P \leq 0.05$) decreased with 6 and 8 g/kg CPLP. The water holding capacity (WHC) of breast muscle was significantly ($P \leq 0.05$) higher with 6 and 8 g/kg CPLP; whereas the robusticity index of tibia bone significantly ($P \leq 0.05$) decreased with 6 and 8 g/kg CPLP inclusion compared to the control group. In conclusion, dietary supplementation of *Calotropis procera* leaf powder (8g/kg) Decreased pH, the muscle fiber diameter of breast muscle and also weight, ash percentage, and density indices of tibia bone whereas increased water holding capacity in Rabbits.

Keywords: bone health, *Calotropis procera*, rabbits, *Oryctolagus cuniculus*, fiber, meat quality, rabbit

The Role of Tm^{3+} Ions in Color Tunable Upconversion Emissions in $CaZrO_3$ Phosphor

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ABSTRACT

Doping of rare earth ions in different combinations and concentrations, to achieve sensitization of luminescence, thermal sensing and anti-counterfeiting color tunable emissions is an emerging field of research. We reported $CaZrO_3: Tm^{3+}$ phosphors for light emitting diodes by adjusting Tm^{3+} sensitizer. High temperature solid state reaction method was used experimentally to achieve calcination of the samples. Increasing the concentration of Tm^{3+} ions enhance the blue emission until the concentration reaches 0.3 mol%. The concentration of Tm^{3+} and the excitation power of 980 nm diode laser have a great effect on the color tunable emission. The temperature-dependent properties of the material indicate that the sample has a good thermal stability. So, this material is beneficial to design multicolor (including white light) displays.

Keywords: Upconversion luminescence, Quenching centers, White light emission, Rare Earth Elements, Phosphors, Luminescent, Light-Emitting Diodes, Thermal Stability, Color Tunable Emission

Phytochemical Analysis of *Salvia moorcroftiana* and its Anti Diabetic Potential in Alloxan Induced Diabetic Mice

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ABSTRACT

Salvia moorcroftiana is a medicinal plant widely used due to their medicinal properties its roots are used in the treatment of colds and coughs. The seed is emetic. It is used in the treatment of dysentery, hemorrhoids, colic and, externally, boils. A poultice of the leaves is used as a dressing for wounds also whole plant is used as anti-diabetic. Diabetes mellitus is a clinical condition characterized by insufficient hyperglycemia due to relative or absolute insulin deficiency or resistance to insulin production at the cellular level. Type 1 diabetes (insulin-dependent), gestational diabetes and type 2 diabetes (noninsulin-dependent) are some types of diabetes. The Type-2 diabetes is the most prevalent type and increases patient population of type-2 DM every year. For preliminary phytochemical analysis the freshly prepared crude methanolic extracts of leaves were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids and alkaloids by using standard phytochemical procedures (Evans *et al.*, 2003). The present study was carried out to investigate the antidiabetic activity and phytochemical analysis of methanolic extract of *Salvia moorcroftiana*. Whole plant extract of *Salvia moorcroftiana* will be administered orally at two doses (150 and 300 mg/kg) to normal and Alloxan induced diabetic mice. Fasting blood glucose (FBG) level, biochemical parameters like blood glucose, serum cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), total protein, urea, creatinine, serum glutamateoxaloacetate, transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) levels and physical parameters like change in body weight, food intake, water intake and levels in liver will be performed for the evaluation of hypoglycemic effects. Both the doses of *Salvia moorcroftiana* caused a marked decrease in FBG levels in STZ induced type 2 diabetic mice. RCO decreased the blood glucose, food intake, water intake, organ weight, serum cholesterol, TG, creatinine, SGOT and sgpt level with significant value and increased the level of HDL cholesterol and total protein with a significant value (<0.05-0.01). The study will provide a positive step in the field of pharmacology.

Keywords: *Salvia moorcroftiana*, Antidiabetic Agents, Phytochemical Analysis, Type 2 Diabetes Mellitus, Hypoglycemic Activity

Occupancy Modelling of the Lizard Specie Saara Hardwickii (Spiny-Tailed Lizard)

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ABSTRACT

Reptiles, like amphibians, are considered an important indicator of environmental health. They are sensitive to changes in their habitat and are used as model animals to understand animal ecology. Documentation of the squamate fauna of Pakistan is satisfactory. However, several species have not been studied in detail. The information on habitat and environmental variables influencing their geographic distribution, occupancy, and habitat association is lacking. We, therefore, conducted the present study to model occupancy and detection probability of *Saara hardwickii* (Spiny-tailed Lizard) and examine site (elevation, substrate, forest type, canopy cover, shrub cover) and survey covariates (time of the survey) affecting their occupancy and detection probability in 36 sites of Southern Khyber Pakhtunkhwa, Pakistan, from September 2022 to May 2023. We ran 18 candidate occupancy models using a package unmarked in R. The occupancy and detection probability of *Saara hardwickii* were 0.78 and 0.70, respectively. The best-fit (null) model did not contribute to the studied site or survey covariates. The study concludes that the studied lizard species are common in parts of KP. The species occur in various habitats (included in this study). *Saara hardwickii* can be detected in the field and their detection is not site/ survey covariate dependent. Our study provides important information on the ecological requirements of this species and their habitat use. We suggest more robust studies incorporating more sites and effects of other survey covariates such as season, observer, and temperature.

Keywords: *Saara hardwickii*, Occupancy, Canopy cover, detection probability.

Isolation and Molecular Characterization of Growth-Promoting Fungal Endophytes from Phoenix Dactylifera

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ABSTRACT

Fungi that reside unnoticeably in plant tissues are known as endophytic fungi. Total, 8 strains were isolated from the roots of Phoenix dactylifera. The isolated endophytic fungal strains from Phoenix dactylifera roots produced sufficient amount of primary, secondary metabolites and growth promoting hormones like IAA, salicylic acids, proline, flavonoids, and phenol etc., which can act as natural biochemical fertilizers and can act in extreme biotic and abiotic stresses inducing resistance both to the stresses as well as disease. Wheat bioassay shows that DT-2, DT-4 and DT-8 are proved as best plant growth promoting endophytic fungal isolates according to better growth response of associated plants (shoot and root length) and production of primary, secondary metabolites and auxin. Highest fresh shoot weight recorded from treated plants was 6.4 g from DT-8 and highest root fresh weight recorded was 1.7g from DT-2. Dt-4 was identified through molecular and phylogenetic approach. DT-4 was identified as a member of Curvularia Lunata. DT-1, DT-2, DT-3, DT-5, DT-6, DT-7 and DT-8 will be identified by phylogenetic analysis in future. In order to maximize plant growth and reduce the need for chemical fertilizers, these fungal endophytes can be used as bio fertilizers to improve production and disease resistance.

Keywords: Endophytic Fungi, Phoenix dactylifera, Plant Growth Promotion, Curvularia lunata, Biofertilizers

Culture Identification and Antibigram of *Klebsiella pneumoniae* Isolated from Clinical Samples

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ABSTRACT

Klebsiella pneumoniae is a Gram negative, non-motile, encapsulated, lactose fermenting, facultative anaerobe belonging to the Enterobacteriaceae family. It is the second most popular member of the aerobic bacterial flora of the human intestine. *Klebsiella* has been associated with different types of infections and one of the most important aspects of the emergence of multidrug resistant strains particularly those involved in nosocomial diseases. This bacterium threatens public health and leads to increased hospital costs and mortality rate. The purpose of this study was to determine the pattern of antibiotic resistance in *K. pneumoniae* in nosocomial infections. Sixteen clinical isolates were collected from Mardan, Khyber Pakhtunkhwa, Pakistan. Most of the samples were collected from sputum, urine and surgical wound site which were followed by biochemical characterization including gram staining, microscopy, oxidase, catalase, Indole and carbohydrate fermentation test. Four confirmed *K.pneumoniae* isolates were further tested for antimicrobial drug sensitivity through KBDD (Kirby Bauer Disk Diffusion Method) and were found to be multidrug resistant. As per CLSI guidelines results of the present study revealed that confirmed *K.pneumoniae* isolates were resistant to Vancomycin 30µg, Gentamicin 10µg, Doxycycline 30µg, Amikacin 30µg, Chloramphenicol 30µg, Amoxicillin 30µg and were susceptible to Piperacillin 100µg and Tazobactam 110µg. These can be a potent antibacterial agent for the treatment of severe bacterial infections caused by *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae*, Gram negative, Multi drug resistant, sputum, antibiotic susceptibility testing.

Invitro Plant Growth Promoting Potential of Sidropore Forming Endophytic Bacteria from *Moringa olifera*

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ABSTRACT

The research explores the in vitro plant growth-promoting potential of siderophore-forming endophytic bacteria isolated from *Moringa oleifera*. *M. oleifera*, a plant renowned for its nutritional and medicinal attributes, harbors a diverse community of endophytic bacteria that may contribute to its overall health and development. The study aims to isolate and identify these endophytic bacteria, assess their siderophore-forming capabilities, and evaluate their impact on the in vitro growth of *M. oleifera*. Bacterial isolates will be obtained from various plant tissues through surface sterilization and molecular identification techniques, focusing on 16S rRNA sequencing. Siderophore production will be quantified through standard assays, providing insight into the iron-acquisition strategies employed by the selected bacteria. Subsequently, in vitro plant growth-promotion assays will be conducted, measuring seed germination rates, root length, and overall plant growth in the presence of siderophore-producing bacteria. The expected outcomes include the identification of endophytic bacteria associated with *M. oleifera*, confirmation of their siderophore-forming capabilities, and demonstration of their in vitro plant growth-promoting effects. The significance of this study lies in its potential to uncover sustainable agricultural practices by harnessing the plant-microbe interactions present in *M. oleifera*. The results may contribute to reducing reliance on synthetic fertilizers and advancing our understanding of the intricate relationships between plants and their associated microbiota. The proposed timeline spans twelve months, covering sample collection, laboratory analyses, and data interpretation, with a corresponding budget allocated for necessary resources. This research endeavors to shed light on the role of endophytic bacteria in enhancing the growth of *Moringa oleifera*, offering implications for agricultural practices that prioritize ecological sustainability.

Keywords: *Moringa oleifera*, Endophytic Bacteria, Siderophores, Plant Growth Promotion, Sustainable Agriculture

Isolation, Molecular Characterization and Plant Growth Promoting Activities of Endophytic Fungi Isolated from *Acacia nilotica*

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ABSTRACT

Facing rising global food demand in a sustainable way is a great challenge of modern agriculture. The increase of crop productivity and resilience in an adverse climate scenario is urgently needed. Fungal endophytes have been described as potential biological tools to improve plant yield and tolerance to biotic and abiotic stresses. Plant-microbe interactions encompass an intricate chemical conversation highlighted by indole-3-acetic acid (IAA) and flavonoids, along with various other signaling molecules. This study investigates the role of IAA, flavonoids and other primary and secondary metabolites in the association between endophytic fungi and wheat plant. Fifteen different fungal strains were isolated from the roots of *Acacia nilotica*, aim to uncover innovative chemical diversity for utilization in agriculture, industry, and pharmaceuticals. In the current study the fungal isolates were checked for the production of bioactive compounds and select those isolates which produce sufficient amount of phytohormones, primary and secondary metabolites. The excellent amount of IAA was produced by AN-1 45.4 µg/ml, and flavonoids from AN-2 are 315.5 µg/ml and proline from AN-3 is 17.71 µg/ml. Wheat seeds were used for bioassay and production of primary and secondary metabolites. The symbiotic association of AN1, AN2, AN3 promoted the growth response of wheat compared with the non-inoculated wheat plants and produce high level of secondary metabolites as compare to control. The inoculated wheat plants enhanced the production of total soluble protein, sugar, lipids, phenolics, flavonoids. Moreover, the plants inoculated with AN-1, AN-2, AN3 enhance the production of total Protein (18%, 20%, 15 %), total soluble sugar (120%, 121%, 105%) and flavonoids (20%, 18 % and 24%) respectively compared with non-inoculated wheat plants. The current study supported the used of endophytic fungi as superior biofertilizers, exerting a significant impact on the agricultural and pharmaceutical sectors owing to their exceptional attributes, including the synthesis of primary and secondary metabolites, as well as phytohormones.

Key Words; Endophytic Fungi, Phenol, Flavonoids, Wheat, Plant Growth Promoting

Exploring the Antioxidant, Anti-Inflammatory and Antidiabetic Potential of Bioactive Myochemical Extract from *Birch maizgill* Mushroom

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ABSTRACT

Researchers from all around the world have been interested in mushroom biomolecules because of their well-established health benefits. These include antioxidant, anti-inflammatory, anti-cancer, antidiabetic. Primary and secondary metabolites are both present in mushrooms. While the secondary metabolite has therapeutic qualities. Verifying the anti-diabetic, anti-inflammatory and antioxidant benefits of *Birch maizgill* polypore mushroom used as traditional remedies to treat diabetes inflammation and oxidative stress was the goal of the current investigation. Antioxidant activity test and in vitro –glycosidase, amylase, yeast uptake and glucose adsorption assays were used to assess the antidiabetic effects. While anti-inflammatory effects assessed by in vitro heat induced and membrane stabilizing activity. All fractions were screened at three different concentrations including 250, 500, 1000 µg/ml were tested for antioxidant, anti-inflammatory and antidiabetic activities. The results shown that fraction F46 demonstrated antioxidant activity, impressively at 84% at 1000 µg/ml. In vitro anti-inflammatory F fraction was most effective showing while the alpha amylase highest inhibitory activity 90%, with F47 consistently exhibiting the highest inhibition alpha glucosidase F47 consistently exhibited the highest inhibitory activity across all concentrations, reaching an impressive 91% at 1000 mg/ml. F46 consistently exhibited the highest glucose uptake inhibition across all concentrations, reaching an impressive 45% at 1000 µg/ml. Conversely, F48 consistently displayed relatively lower glucose uptake inhibition across the tested concentrations. F36 showing the highest glucose adsorption a diverse range of anti-inflammatory activities was observed, with F60 displaying the highest inhibition at 1000 µg/ml. F47 consistently demonstrated the highest hemolysis protection activity at this concentration, while F12 consistently exhibited relatively lower activity. Our results concluded that ethanolic extracts from *birch maizgill* can be used as effective antioxidant, antidiabetic and anti-inflammatory potential. Future research can be conducted in vivo studies and liquid chromatography mass spectrometry (LCMS) will help in identification of specific bioactive compounds.

Keywords: *Birch maizgill*, bioactive compounds, antidiabetic, antioxidant, anti-inflammatory

Prevalence of Gestational Diabetes Mellitus and its Associated Risk Factor in District Malakand

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ABSTRACT

Gestational diabetes mellitus is the type of diabetes mellitus which occur during pregnancy and diabetes mellitus is a condition in which the blood glucose level increases pathologically. The 207 women were including in the study for data collection. Data were collected using questionnaire, laboratory investigation report. In the total 207 consents 26(13%) were GDM positive and most commonly occurring in the 24-56 age pregnant women, house wives. The gestational diabetes is a significant factor to monitor in pregnant women because it affects the number of pregnant women.

Keywords: Gestational Diabetes Mellitus, Pregnancy

Effect of Root Exudates on Spore Germination and Mycelial Growth of Different Endophytic and Pathogenic Fungi

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ABSTRACT

Root exudates have the potential to reshape microbial communities in the rhizosphere by differentially attracting or repelling microbes. Current study was conducted to understand the potential of root exudates to inhibit or promote fungal spore germination. Total 6 fungal strains were isolated from plants collected from the premises of Abdul Wali Khan University Mardan. Out of them 3 were endophytes isolated from *Triticum aestivum* L. The rest of the 3 isolates were pathogenic from *Lycopersicon esculentum* L. On the basis of DNA barcoding (based on ITS of 18 S rRNA gene) endophytic strains were identified as *Aspergillus terreus*, *Aspergillus fumigatus* and *Aspergillus awamori*. Similarly, pathogenic strains were identified as *Aspergillus tamarii*, *Aspergillus oryzae* and *Aspergillus niger*. All isolated strains were subjected to root exudates (inoculated by pathogen and endophytes and un-inoculated) of host and non-host plants. Spores of endophytic strains showed equal germination toward both host and non-host plant root exudates. But spores of pathogenic strains showed no germination toward its un-inoculated host plant's root exudates. The effect of inoculated plant's root exudates was very different from un-inoculated, the former being promoting the germination of previously dormant spores of pathogens when induced by pathogen. Contrary to this, root exudates from endophytes inoculated seedlings enhanced spore germination in endophytic strains and inhibited in the pathogenic strains. Similar pattern was seen in case of fungal hyphal growth as well. Interestingly, it was found that tomato exudates of roots (un-inoculated) germinated only secondary spores of endophytes while wheat germinated only conidia as primary spores. So it may be concluded that endophytes are attracted and pathogen are inhibited root exudates of plants (un-inoculated). Pathogen are only attracted when plants are already inoculated by pathogen by some others ways away from exudates attraction.

Keywords: Inoculated vs Un-inoculated, Mycelial growth; Root exudates; Spore germination.

Effect of Melatonin on Pathogenesis Related Gene Expression in Invitro Potato

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the fourth most essential crop in the world. It produces tremendous amount of carbohydrates and plays important role in human diet which comprises of 18% carbohydrates, 2 to 3% of protein and 80% of water. Melatonin is a natural pleiotropic molecule, referred as N-acetyl-5-methoxytryptamine, is widely present in animals, plants, and microbes. Melatonin can induce plant innate immunity against pathogen infection, but the direct effects of melatonin on plant pathogens are poorly understood. Melatonin significantly regulates various abiotic stresses. In current study, the direct effects of melatonin on *P. Infestans* was investigated. Methoxytryptamine, is widely present in animals, plants, and microbes. Initially, its function is known to regulate circadian rhythms. Subsequently, other functions including the modulation of mood, sleep, metabolism, and antioxidant are also established in diverse organisms. Specifically, melatonin has been reported to be effective in inhibiting the cell growth in some human pathogens by impairing their mitochondrial functions, inhibiting biofilm formation, and reducing intracellular substrates. In the current study, the effects of melatonin, a major regulator of plant development and stress response, on the expression of genes related to pathogenesis was investigated. The current results provide light on melatonin's complex participation in cellular responses and point to a potential role for it in modifying the plant's defense mechanisms. By shedding light on melatonin-mediated gene regulation in potatoes. Furthermore, Melatonin has been found to increase crop yield. It enhances the activity of carbohydrate and nitrogen metabolism.

Keywords: potato, melatonin, PR gene

Stripe Rust Biological Control by Fungal Endophytes and Elucidation of Resistance in Diverse Wheat Varieties

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ABSTRACT

Wheat (*Triticum aestivum* L.) is an important cereal crop of Pakistan and has a significant impact on the economy. Several abiotic and biotic factors affect the yield of wheat, which results in less production of wheat. The stripe rust caused by *Puccinia striiformis* is an important wheat disease in many countries of the world as well as in Pakistan. Salicylic Acid, Jasmonic Acid, and Ethylene are phytohormones that regulate plant defense responses to biotic stresses. SA is primarily responsible for resistance to hemi bio trophic and bio trophic pathogens in Arabidopsis, the model plant. JA and ET, on the other hand, are primarily involved in resistance to necrotrophic pathogens. Use of endophytes is gaining more attention for the protection of plants from different stresses. In the current study, entophytic fungi, isolated from *Phoenix dactylifera* which were able to produce sufficient amount of plant growth promoting hormones, Primary and secondary metabolites. Anti-*Puccinia graminis* activity of fungal endophytes was assessed by quantifying the presence or absence of inhibition zone in dual cultures. Selected entophytic fungi were evaluated for their ability to inhibit the growth of *Puccinia striiformis*, and evaluated for activities of hydrolytic enzymatic activities. Wheat plants were infected to check the disease suppression response induced by entophytic fungi. Disease resistance response of Wheat plants (inoculated with endophytes and infected with *Puccinia striiformis* in comparison to the controls) was evaluated by quantifying antioxidant enzyme activities (PAL, PPO, CAT, SOD, APX and POX) and the expression of Yr5, Yr7, Yr8, Yr10, Yr15, Yr17 and Yr18 genes were used in RT-qPCR. Level of malondialdehyde, H₂O₂, relative electrolytic leakage and disease index was calculated. The current study supported the use of entophytic fungi for biological control of stripe rust and used as biofertilizer in disease condition.

Keywords: Endophytic fungi, Stripe rust, Phenolic compounds, proline, Anti-oxidant

Genome-Based Reclassification of *Fontibacillus solani* and *Fontibacillus panacisegetis* Species in the Family *Paenibacillaceae*

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ABSTRACT

The genus *Fontibacillus* is a member of the family *Paenibacillaceae*, of the order *Bacillales*, and class *Bacilli*. Taxa from the genus *Fontibacillus* are catalase-negative and oxidase-positive. The genus *Fontibacillus* includes five species. Objective of current work was to use bioinformatics tools, which provide a less expensive and more rigorous method to get genomic data, to elucidate the taxonomic relationship among *Fontibacillus solani* CECT 8693^T and *Fontibacillus panacisegetis* KCTC 13564^T. The sequence of 16S rRNA of *Fontibacillus solani* CECT 8693^T and *Fontibacillus panacisegetis* KCTC 13564^T were compared, and the results revealed a high degree of similarity 97.71% through Joint Genome Institute. *Fontibacillus solani* CECT 8693^T and *Fontibacillus panacisegetis* KCTC 13564^T are shown to be closely related within the genus *Fontibacillus*, according to phylogenetic dendrograms construct by using the MEGA X Software based on the sequence of 16S rRNA gene and we also carried out to determine these two strains precise taxonomic positions on genome-based analysis by using the MEGA X Software. Assessment of the *Fontibacillus solani* CECT 8693^T and *Fontibacillus panacisegetis* KCTC 13564^T sequences showed a high similarity value of 76.80% through the Genome-to-Genome Distance Calculator and declared these two strains belonged to the same species. Also, an examination of their phenotypic characteristics from the articles of their respective species revealed that there were no significant phenotypic differences between *Fontibacillus solani* and *Fontibacillus panacisegetis*. Considering these data, we propose that *Fontibacillus solani* be categorised as a later heterotypic synonym of *Fontibacillus panacisegetis*.

Keywords: *Fontibacillus*, *Paenibacillaceae*, *Bacillales*, *Bacilli*, bioinformatics tools, *Fontibacillus panacisegetis*, *Fontibacillus solani*

Bioremediation of Waste Water by the Use of Aquatic Plants

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ABSTRACT

One of the main factors contributing to pollution and environmental deterioration is the growing urbanization, industrialization, and population. Water pollution is considered a leading source of environmental contamination, especially heavy metal pollution in ground water poses a serious threat to human health and the aquatic ecosystem, including lead, zinc, cadmium, and arsenic. Heavy metals and many such pollutants have detrimental impacts on all living things. A deadly contaminant that upsets the food chain and is lead, Untreated waste water is widely used for agriculture in many countries. This is one of the world leading serious environmental and public health concerns The current purification methods for eliminating pollutants from wastewater are not only exceedingly expensive, but they also have a detrimental effect on the environment, Conventional treatment technologies to remove the pollutants from wastewater are usually costly, time consuming, environmentally destructive, and mostly inefficient. Moreover, environmental toxicity due to solid waste exposures is also one of the leading health concerns. Bioremediation. Phytoremediation is a coeffective green emerging technology with long lasting applicability. The purpose of this research was to assess the current state of bioremediation as an innovative technology and to discuss its usefulness and potential in the remediation of contaminated water. The selection of plant species is the most significant aspect for successful phytoremediation. Aquatic plants hold steep efficiency for the removal of organic and inorganic pollutants. Water hyacinth (*Eichhonia crassipies*), water lettuce (*Pistia stratiotes*), vetiver grass (*Chrysopogon zizanioides*), Duck weed (*Lemna minor*) were used to treat the waste water, Among which the water hyacinth and vetiver grass were considered as the best aquatic plants for the treatment of waste water.

Keywords: bioremediation, phytoremediation, heavy metals, pollutants, aquatic plants, waste water, remediation

Evaluation of *Salvia hispanica* Seeds as Source of Potent Bioactive Carbohydrates

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ABSTRACT

Natural mucilage and gums isolated from plant have been widely used in different capacities including as excipient, therapeutic formulations, intensifying agent, gelling agent increase food shelf life and other functional food products. Chia seeds release high amount of mucilage that have several bioactive role. Chia seed is an annual herbaceous plant came from Mexico and Northern Guatemala. This study was aimed to optimize the process of mucilage extraction and to fractionate and evaluate the biological role of polysaccharides present in the chia seed mucilage. Mucilage was extracted and dried with the help of a lyophilizer and the dried mucilage was fractionated in 75% v/v ethanol. Biological activities of crude and each fraction was determined using various biological assays. The antimicrobial activity was performed using well agar well diffusion method. The results revealed that among the screening fraction ethanol precipitated fraction was the most potent one which induce 3.5mm zone of inhibition in *S. typhi*, 5.2mm in *S. aureus* while no significant zone of inhibition was observed in crude fraction. Radical scavenging activity of the test samples were determined using DPPH where ethanol non-precipitated fraction was found to have the best IC₅₀ value that is 536 µg/mL followed by ethanol precipitated fraction 570 µg/mL. Alpha amylase inhibition of the extract was determined through standard alpha amylase inhibition assay where ethanol non-precipitated fraction showed an IC₅₀ value of 546 µg/mL. Ethanol non-precipitated fraction was found to significantly reduce the paw edema in carrageenan induced paw edema mice model. The results concluded that among all the chia seed mucilage fraction, ethanol non-precipitated fraction exhibited significant biological activity which could be further screened and investigated for potential antidiabetic drug development. The evidence resulted in this study will further strengthen the search of other plant based-mucilage and their development as alternative therapeutic measurements. Furthermore, biochemical and molecular characterization of these mucilage fractions will understand and increase our knowledge behind the exact molecular mechanism involved in its bioactivity.

Keywords: *Salvia hispanica*, chia seeds, bioactive carbohydrates, mucilage extraction, polysaccharides, *S. typhi*, *S. aureus*

Investigating the Association of two Variants rs5743899 and rs3750920 of Human Tollip Gene with Cutaneous Leishmaniasis

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This study was performed to examine two single nucleotide polymorphisms of TOLLIP gene which are located in intron-4 and exone-4. The SNPs involved in this study was (rs5743899 and rs3750920). A total of 400 individuals included 200 infected patients of Leishmaniasis and 200 healthy were selected for this study. The total samples were genotyped for the SNPs (rs5732899 and rs3750920) of TOLLIP gene using chain restriction-fragment length polymorphism (PCR-RFLP) method. From the genotypic frequency analysis of both variants the following values were obtained. TOLLIP gene variant rs5743899 genotypic frequency of AA genotype was in case 120 (60.0%) as compared to control 92 (46.0%), AG genotype 70 (35%) were in cases and 84 (42.0%) were in control sample similarly GG genotype in cases 10 (5.0%) as compared to GG genotype of control which is 24 (12.0%). Statistical value for this variant are χ^2 (10.74), df (2), and p-value is (0.047). Such like that for the second variant of TOLLIP gene rs3750920 genotype frequency of AA were in cases 72 (36.0%) while in control sample 98 (46.0), AG were in case 92 (46.0%) and in control 86 (43.0), similarly the GG genotype frequency in cases 36 (18.0%) and in control sample 16 (8.0%). Statistical value for this variant are χ^2 (11.87), df (2), and p-value is (0.00026). From the allelic frequency analysis for TOLLIP gene variant rs5743899 the allelic frequency for A and G allele is almost same in both control and patients (p=0.0012, OR 1.697 (1.239-2.332), RR 0.9754 (1.061-1.262). Similarly for the second variant rs3750920 the frequency for A and G allele were also same in both control and cases (p=0.0009, OR 1.661, RR 1.195). The G and T allele were consider as carrier dominant allele because evidence were found for the dominant or recessive mode. Based on these finding we can say that these two polymorphisms of TOLLIP gene are associated with the increased risk of developing cutaneous Leishmaniasis.

Keywords: Tollip Gene, SNP, Cutaneous Leishmaniasis

Evaluation of Resistance Potential of Carbon Nanotubes-Induced Invitro Potato Plants against Bacterial Pathogens

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ABSTRACT

The study aimed at determining the effects of carbon nanotubes (CNTs) on potato morphological parameters and biosynthesis of biochemical compounds. We used different concentrations of CNTs (10-80 mg/l) for callus multiplication and then its regeneration. The in vitro plants of potato (cv. Desiree) were multiplied on basic MS medium added with 2.0 mg/l GA₃. Inter-nodal stem cuttings and meristematic shoots were separated with a sterilized cutter (surgical blade) and cultured on the MS Media containing 2% sucrose, 1.0% agar and 2.0 mg/L 2, 4-D, and different concentrations of CNTs was used for the calli initiation. Data was recorded for callus total fresh and dry weight, number of shoot and root, and length of shoots and roots. Similarly, the calli cultures were subjected to spectrophotometric analysis for the determination of different biochemical compounds such as total flavonoids, phenolic acids, proline and carotenoids. In the last, antioxidant and antimicrobial activities of the compounds was checked using DPPH assay, while the antibacterial activity was determined against two pathogenic strains *R. solanacearum* and *E. carotovora* using disk diffusion method. Our investigation showed that high concentrations of the CNTs stimulated plant growth also the level of biochemical compounds in potato. The calli initiation percent, the number of shoots, the roots per callus, and the callus biomass all enhanced dramatically, when the CNTs were employed in place of PGRs. Similarly amount of total biochemical contents were also found to be increased at highest concentration that showed antioxidant activity and maximum inhibitory activity against tested pathogens.

Keywords: Carbon nanotubes, callus induction, regeneration, biochemical compounds.

Phytoremediation of Domestic Waste Water Using Aquatic Plant *Water Hyacinth*

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ABSTRACT

Water, as a basic human need, is experiencing a global scarcity crisis, due to rapid depletion of natural water supplies and ground subsurface water contamination. Domestic sewage is a major source of water pollution, with a significant impact on surface water bodies. As a result, wastewater must be properly treated before being released into the environment. Conventional treatment technologies are currently ineffective in removing all toxins from water. Phytoremediation is a technique that employs the application of plants for the remediation of wastewater. Aquatic plants have the capacity to absorb excess contaminants such as organic and inorganic, heavy metals, and pharmaceutical pollutants present in domestic wastewater. Phytoremediation of domestic wastewater was done using an aquatic plant water hyacinth (*Eichhornia crassipes*). Experiments were performed in a plastic container with a volume of 11 inch length and 12 inch width. 15 liter domestic wastewater was used for the culture of water hyacinth. The domestic wastewater quality was assessed by analyzing physicochemical parameters which are temperature, pH, total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), nitrate, alkalinity, phosphate, oil and grease of a domestic wastewater before phytoremediation, after 7 days and after 14 days of treatment. Different parameters show gradual decrease after the treatment. The result showed a promising decrease in all the parameters in which total suspended solids and phosphate showed the highest reduction. In the present work an attempt has been made to remove pollutant load of domestic wastewater by using phytoremediation technique. Thus, the results of physicochemical analysis of waste water have suggested that Water hyacinth can be used for the purpose of phytoremediation.

Keywords: phytoremediation, domestic waste water, aquatic plants, water hyacinth

Health Potential of Bioactive Polysaccharides from Okra

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ABSTRACT

Plant derived mucilage, due its role in promoting human health, has been extensively used as active constituent for the preparation of different pharmaceuticals, functional and nutraceutical products. Mucilage mainly consists of complex carbohydrates with extremely branched structure. *Abelmoschus esculentus L.* (Moench), belongs to family Malvaceae, is an annual herb commonly known as okra. The purpose of this study was the identification of bioactive polysaccharides present in the mucilage of okra pods and evaluation of their biological activities. Mucilage was extracted by soaking okra pods in water. The dried mucilage was fractionated in 75% ethanol, which resulted in soluble and insoluble fractions. Each fraction was screened which confirmed the presence of galacturonic acid, saturated and unsaturated polysaccharides. Crude, soluble and insoluble fractions were processed for investigating their biological activities. The results indicated that insoluble fraction was able to inhibit the growth of five human pathogenic bacterial strains i.e. *Staphylococcus aureus* ($1.9 \text{ mm} \pm 0.27\text{mm}$), *Escherichia coli* ($2.8\text{mm} \pm 0.44\text{mm}$) and *Shigella* ($2.9\text{mm} \pm 0.85\text{mm}$). However no significant inhibition was observed against *Klebsiella pneumonia* and *Salmonella typhi*. Antioxidant capacity was determined through DPPH assay where the soluble fraction showed IC₅₀ value $785.5 \mu\text{g/mL}$ while the insoluble fraction showed IC₅₀ value $987.21 \mu\text{g/mL}$. Alpha amylase inhibition assay results of the extract showed that soluble fraction has IC₅₀ value $451.63 \mu\text{g/mL}$, followed by insoluble and crude fraction having IC₅₀ value $767.13 \mu\text{g/mL}$ and $1152.61 \mu\text{g/mL}$ respectively. In vivo anti-inflammatory effect of the fractions was determined by carrageenan induced paw edema in mice model. The result revealed that soluble fraction at 800 mg/kg was able to reduce the paw edema volume from $4.81\text{mm} \pm 0.18\text{mm}$ to $2.82 \text{ mm} \pm 0.12\text{mm}$ which was comparable to standard drug diclofenac sodium ($2.12 \text{ mm} \pm 0.24\text{mm}$). Our results conclude that okra pods exude contains potential bioactive polysaccharides. However, further molecular identification and characterization of these bioactive polysaccharides will further broaden our knowledge of the important roles played by these compounds in human health and nutrition.

Keywords: Bioactive Polysaccharides, Okra, *Abelmoschus esculentus*, Moench, *S. aureus*, *E. coli*, *Shigella*, *K. pneumonia*, *S. typhi*

Effect of Temperature on Alpha-Amylase Producing Bacteria Isolated From Food Waste and Soil

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ABSTRACT

Alpha-amylase is a starch degrading enzyme that can be obtained from different sources such as plants, animals, and microorganisms. But due to ease of isolation and maximum production rate of enzymes microbial sources are preferred over other sources. Among microbial sources, bacteria and fungi are mostly used for the production of α -amylase both at small and industrial scales because they are generally cheaper to produce their enzyme contents, and also the process of extraction and purification of enzymes from microbial source is easier in comparison with plants and animal sources. *Bacillus*, *Pseudomonas*, and *Staphylococcus* strains are mostly preferred for the production of α -amylase because it has a short generation time and its environmental and genetic manipulation is easy. In the current project, 29 different bacterial strains were isolated on nutrient agar media from food wastes. Samples were collected from Gujar Garhi and Sawabi. All strains were screened in which six bacterial strains (*Bacillus subtilis*, *Bacillus subtilis*, *Pseudomonas* sp., *Pseudomonas leutola*, and *Staphylococcus*) were producers of α -amylase and selected for further experimentations on the basis of clearance zones (4.5 mm, 4 mm, 3.125 mm, 6.33 mm, 2.5 mm, 3.5 mm), with the largest zone produced by *P. leutola* ZS4. The bacterial strains were further characterized by gram staining and biochemical tests. Among six positive producers, best strain was selected for further analysis. on the basis of a comparatively larger clearance zone. Furthermore, the effect of different temperatures was evaluated for the maximum production of α -amylase produced by *P. leutola* ZS4 and maximum units of enzymes (1.03715) were produced at a temperature of 40 °C. The current study supported the used of *P. leutola* ZS4 for the efficient production of second-largest group of enzymes α -amylase in the world market and found potential applications in different industries.

Keywords: Amylase, *Bacillus*, food waste, *Pseudomonas*, *Staphylococcus*, DNS, enzymes

Differential Proteomic Analysis of *H. Pylori* Isolated From Gastric Patients

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ABSTRACT

Helicobacter pylori is a human stomach-dwelling organism that causes many gastric illnesses, including gastritis, ulcer, and gastric cancer. The purpose of the study was to perform differential proteomic analysis on *H. pylori*- isolated from gastric biopsies of infected individuals with gastritis, ulcer, and gastric cancer. Differential proteins were identified by making pooled samples of each group and subjected to nano-LC-QTOF MS/MS analysis. A total of 44 significantly regulated proteins were identified in *H. pylori* isolated from patients with either gastritis, ulcer, or cancer. Comparative analysis of groups revealed that in case of cancer vs gastritis, 16 proteins were over expressed out of which 9 proteins including Cag3 & FlhA were upregulated in only isolates from cancer patients. Similarly in cancer vs ulcer a total of 19 proteins were expressed. DNA Helicase & Adenyl succinate lyase were upregulated in cancer while Cag3 and FlhA were downregulated. In ulcer vs gastritis, 10 proteins were expressed. In this group, FlhA was overexpressed. A reduction in DNA gyrase subunit B was observed in ulcer vs gastritis and cancer vs ulcer. Our study suggested Six discrete protein signatures, Cag3, FlhA and DNA gyrase subunit B with differential expression in gastritis, ulcer, and cancer. Protein expression profiles of *H. pylori* isolated from patients of these gastric diseases will help to understand the virulence and pathogenesis of *H. pylori*.

Keywords: Differential proteomics, Gastritis, *H. pylori*, Mass-spectrometry

Variability and Control Study of Apple Scab Fungus

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ABSTRACT

Apple scab is a disease of Malus tree that is caused by the ascomycete fungus, *Venturiainaequalis*. The ailment shows as dim dark or dull darker lesions on the external of tree buds, leaves or fruit. Colony diameter of *V. inaequalis* and antifungal activity of garlic was carried out. Different locations of Swat, Kalam and Chitral were selected for the study. The most aggressive growth was shown by the isolate B1 (7.1cm), N8 (6.5cm), P7 (6.4cm), G1 (6.2cm) and C1(3.9cm). Garlic extract was used for finding the inhibition of the most aggressive isolate of *Venturia inaequalis*. Five different garlic concentrations were prepared in this regard i.e. five percent, ten percent, fifteen percent, twenty percent, and twenty five percent. These different concentrations were applied on most aggressive isolates and their antifungal activity was measured. Findings were quite impressive showing maximum inhibition of *Venturia Inaequalis*. 5 percent concentration of garlic showed maximum inhibition of 1.8cm for sample N8 while 10 percent concentration showed maximum inhibition of 2.2cm for sample G1. For 15 percent maximum inhibition was 1.7cm for most aggressive isolate of sample B1 while 20 percent showed maximum inhibition of 1.8cm for sample P7 and 25 percent concentration show maximum inhibition of 1.5cm for sample C1. Statistical analysis was carried out using SPSS package (version 20). Analysis of variance technique was used for finding any significance differences between the mean isolates of *V. inaequalis* of different areas. ANOVA test showed significant level at 0.05. Multiple comparison test results showed that Chitral means is significantly different from other isolates means. Significant level for Biha and Chitral was 0.006, Nilkot and Chitral was 0.024, Pashtunai and Chitral was 0.029, and Chitral and Gwalaria were 0.001. Multiple Comparisons for garlic extract concluded significant level for Biha and Nilkot 0.046, Biha and Gwalaria 0.000, Nilkot and Gwalaria 0.000, Nilkot and Chitral .009, Pashtunai and Gwalaria 0.000, Chitral and Gwalaria 0.000.

Keywords: Apple scab, Malus tree, Ascomycete fungus, *Venturiainaequalis*

Unraveling HIV Susceptibility: A Comprehensive Analysis of TLR4 and TLR7 Polymorphisms in the Pakistani Population with Insights into Phytochemical Therapeutics

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ABSTRACT

Genome-wide association studies (GWAS) play a pivotal role in investigating the correlation between genetic variants and the predisposition to specific diseases. This research avenue not only facilitates the customization of targeted therapies according to an individual's genomic makeup but also enables the identification of genetic markers for early disease detection. In this context, Toll-Like Receptor-4 (TLR4) polymorphism (rs45986790) and TLR7 polymorphism (rs179008) frequencies in HIV patients have been extensively explored in European and American populations. However, a comprehensive study of these polymorphisms in the Pakistani population remains absent. This study addresses this gap by examining the prevalence of TLR4 and TLR7 genetic variants in a cohort of 50 samples, including 38 confirmed cases of HIV and 12 confirmed cases of AIDS, collected from diverse regions in Punjab. Additionally, pharmacogenetic analysis involving both phytochemicals and FDA-approved antiretroviral drugs is conducted to assess alterations in drug response attributed to Single Nucleotide Polymorphisms (SNPs). An in-silico docking study further investigates the binding affinities of drugs with both normal and mutant proteins. Results indicate that the frequency of the TLR7 variant (minor allele T) rs179008 is not statistically significant ($p=0.1$), and the TLR4 variant (minor allele G) is not detected. Notably, two phytochemicals, Chlorogenic acid and Glycyrrhisoflavone, exhibit the highest binding affinities with mutant proteins, comparable to FDA-approved antiretroviral drugs. In conclusion, this study reveals that polymorphic variants of TLR4 and TLR7 are not associated with susceptibility to HIV. Furthermore, the identified phytochemicals, characterized by their high binding affinities, underscore the potential application of phytochemicals in the treatment of chronic infections such as HIV.

Keywords: HIV, Toll-Like Receptors, Single Nucleotide Polymorphisms, Pharmacogenetic Analysis, Phytochemicals

Impact of Foliar Application of Bio-Stimulants on Yield and Quality Attributes of Fodder Oat

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ABSTRACT

A field experiment conducted at the National Agricultural Research Center in Islamabad, the influence of bio-stimulants on oat fodder yield and its associated parameters was investigated. The experiment involved three factors: Moringa leaf extract (0%, 5%, 10%), Sea weed extract (0%, 2%, 4%), and Chitosan (0 ppm, 100 ppm, 200 ppm). The study was designed using a Randomized Complete Block Design (RCBD) with three replications. Significantly higher values were observed for various parameters when using Moringa leaf extract at a concentration of 10%. This included greater plant height (114.1 cm), leaves per tiller (6.2), tillers per plant (17.1), leaf area index (8.9), internode length (16.4 cm), stem girth (6.4 mm), plant fresh weight (184.0 g), plant dry weight (109.1 g), green fodder yield (64.3 t/ha), dry matter yield (14.0 t/ha), photosynthetic rate (10.4), respiration rate (6.1), CO₂ emissions (6.1 kt), transpiration rate (6.0 g/m²s), and evapotranspiration (5.7 MJ/m²/day). For Sea weed extract at 4%, there were also significant improvements in several parameters, such as plant height (113.9 cm), leaves per tiller (6.3), tillers per plant (15.3), leaf area index (8.7 cm), internode length (16.4 cm), stem girth (6.3 mm), plant fresh weight (177.0 g), plant dry weight (99.0 g), green fodder yield (60.5 t/ha), dry matter yield (12.6 t/ha), photosynthetic rate (10.2), respiration rate (5.0), CO₂ emissions (4.4 kt), transpiration rate (4.5 g/m²s), and evapotranspiration (4.5 MJ/m²/day). Chitosan at 200 ppm led to the maximum values for plant height (114.3 cm), leaves per tiller (6.2), tillers per plant (16.0), leaf area index (8.9 cm), internode length (16.9 cm), stem girth (6.5 mm), plant fresh weight (185.4 g), plant dry weight (102.0 g), green fodder yield (58.5 t/ha), dry matter yield (12.5 t/ha), photosynthetic rate (10.2), respiration rate (5.2), CO₂ emissions (5.2 kt), transpiration rate (5.3 g/m²s), and evapotranspiration (5.3 MJ/m²/day). The control group recorded lower values for these parameters. In conclusion, the study demonstrates that the application of Moringa leaf extract at 10%, Seaweed extract at 4%, and Chitosan at 200 ppm significantly enhances oat fodder yield and its associated parameters. Therefore, it is recommended to use these concentrations as a best practice for improving oat fodder yield.

Keywords: Oat, Biostimulant, Moringa leaf extract, Sea weed extract, Chitason.

Seroprevalence and Risk Factors Associated With Crimean Congo Hemorrhagic Fever Virus (CCHFV) in Human and Livestock Population in District Malakand

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ABSTRACT

Crimean Congo Hemorrhagic Fever (CCHF) is a viral zoonotic tick-borne disease mainly caused by CCHF virus and distributed in domestic and wild animals. The transmission of CCHFV is mainly occurs via bite of tick (*HylomaSp*) to human and animals. The present study was performed to determine the seroprevalence of IgG antibodies to CCHF in human and livestock's population and associated risk factors among both populations. A total 10 union councils were selected in District Malakand in which 54 households/ Farms were visited. Total 541 serum samples were collected from both populations. All farmers were extensively interviewed to identify major risk factors. A total 401 serum samples were screened on ELISA for the presence of IgG antibodies in both Populations. A total 29.1% prevalence was recorded in livestock population. Highest ratio was detected in tehsil Batkhela (36.6%). Lowest ratio was recorded in tehsil Dargae. No positive sample was detected in farmers. Goat was highly exposed to CCHFV in present study (43%). Among the major risk factors of CCHF, it was observed that *Hyloma* ticks being circulating in all households are on the top. All individual were in closed contact with livestock. Most of the participants were unaware of CCHF and its transmission which increased risk of CCHF. On the basis of above facts it is concluded that there is a high ratio of CCHF in livestock population. If proper preventive measures were not adopted on time, it may cause a huge outbreak.

Keywords: Crimean Congo Hemorrhagic Fever Virus (CCHFV), Seroprevalence, Cross-sectional, Risk Factors, Livestock, Human.

Synthesis, Characterization, Hirshfeld Surface Analysis, Antioxidant and Selective B-Glucuronidase Inhibitory Studies of Transition Metal Complexes of Hydrazide Based Schiff Base Ligand

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ABSTRACT

The synthesis of 2-amino-N'-[(E)-(4-hydroxy-3-methoxyphenyl) methylidene] benzohydrazide (HAHMB) was performed by condensing O-vanilin with 2-aminobenzohydrazide and was characterized by FTIR, high resolution ESI(+) mass spectral analysis, ¹H and ¹³C{¹H}-NMR. The compound H-AHMB was crystallized in orthorhombic Pbc_a space group and studied for single crystal diffraction analysis. Hirshfeld surface analysis was also carried out for identifying short interatomic interactions. The major interactions H...H, O...H and C...H cover the Hirshfeld surface of H-AHMB. The metal complexes [M(AHMB)_n] where M = Co(II), Ni(II), Cu(II) and Zn(II) were prepared from metal chlorides and H-AHMB ligand. The bonding was unambiguously assigned using FTIR and UV/vis analysis. The synthesized ligand H-AHMB and its metal complexes were studied for β-glucuronidase enzyme inhibition. Surprisingly the metal complexes were found more active than the parent ligand and even the standard drug. Zn-AHMB shown IC₅₀ = 17.3±0.68 μM compared to IC₅₀ = 45.75±2.16 shown by D-saccharic acid-1,4-lactone used as standard. The better activity by Zn-AHMB implying zinc based metallodrug for the treatment of diseases associated with β-glucuronidase enzyme. The DPPH radical scavenging activities were also studied for all the synthesized compounds. The Co-AHMB complex with IC₅₀ = 98.2±1.78 μM was the only candidate to scavenge the DPPH free radicals.

Keywords: 2-Aminobenzohydrazide, Metal Complexes, β-Glucuronidase Inhibition, DPPH Radical Scavenging, Zinc Metallodrugs, Hirshfeld Surface Analysis, Orthorhombic Crystallography

The Role of Tm^{3+} Ions in Color Tunable Upconversion Emissions in $CaZrO_3$ Phosphor

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ABSTRACT

Doping of rare earth ions in different combinations and concentrations, to achieve sensitization of luminescence, thermal sensing and anti-counterfeiting color tunable emissions is an emerging field of research. We reported $CaZrO_3: Tm^{3+}$ phosphors for light emitting diodes by adjusting Tm^{3+} sensitizer. High temperature solid state reaction method was used experimentally to achieve calcination of the samples. Increasing the concentration of Tm^{3+} ions enhance the blue emission until the concentration reaches 0.3 mol%. The concentration of Tm^{3+} and the excitation power of 980 nm diode laser have a great effect on the color tunable emission. The temperature-dependent properties of the material indicate that the sample has a good thermal stability. So, this material is beneficial to design multicolor (including white light) displays.

Keywords: Upconversion luminescence, Quenching centers, White light emission

Evaluation of Genotoxic Effects of Homeopathic Medicines (*Lycopodium clavatum* and *Nux vomica*) Through Comet Assay and Micronucleus Assay

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ABSTRACT

Practicing homeopathic or complementary treatment have attained substantial popularity across the world due to the holistic approach and reduced side effects attributed to its slow action. However, a controversy arises over the utilization of large quantity of alcohol in making homeopathic potencies. The aim of present research study was to assess the potential genotoxicity of *Lycopodium clavatum* and *Nux vomica* on human lymphocytes *in vitro* and Balb-C mice *in vivo* via Comet assay and bone marrow micronucleus assay. Effect of Mother tincture (Lyc. Q and Nux. Q) as well as two potencies (Lyc.30, Lyc.200 and Nux.30, Nux.200) of the both the drug were evaluated for time interval 12 hrs, 18 hrs and 24 hrs and 7days, 15days and 21 days for *in vitro* and *in vivo* study respectively. Tail DNA %, Tail length, Tail moment and Olive moment were selected as parameters for calculation of extent of DNA damage. Data obtained from the present study showed that higher potency of both drugs have more toxic effect on DNA as compare to their mother tincture, that is Lyc.200 C (Tail DNA % (64.36 ± 1.03) showed more damage, followed by Nux.200C (tail DNA 52.24 ± 9.61), while low level damage was caused by mother tincture of both drugs. However, in contrary to expectation, no considerable damage was caused by positive control. So it can be assumed that damage was caused upon binding with drug extract in excess dilution of alcohol. Further study is considered necessary to find the mechanism of action these homeopathic medicines.

Keywords: Homeopathy, Mother tincture, Comet assay, genotoxicity, micronucleus test

Distribution, Food Preference, and Threats to *Alectoris chukor* in District Karak, KP Pakistan

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ABSTRACT

This study aimed to explore and enlist the *Alectoris chukar* and their diversity in the study area Karak district, KP, Pakistan, to gather information about the population distribution, presence, major threat, and food preference and habitat utilization during the period from May to November 2023. In Pakistan it occurs in a wide range of rough, sloping and dry regions rising to the higher foothill valleys of the internal Himalayan ranges and western Himalayas and plains, Balochistan, and dry hillsides of the Punjab and Sindh. It is the “National Bird” of Pakistan, however, very little is known about its feeding preference and mode of distribution in its native range in the north of the country. Primary data was collected through visits to the study areas in the morning and late afternoon along with using binoculars, Cameras, Geographical Positioning systems (GPS), Wooden sticks, Field bags, Survey data sheets, Survey permits etc. To study its habitat and their different activities in the study areas through questionnaire survey. While secondary data was collected from available literature. Population distribution based on dividing the whole area into four zones which were further subdivided into different localities. Zone-A mountainous stretch of Wanki Siraj Khell to Takht E Nasrati with edge ways mountain belt from Chukara to the plains area of Ahmed Abbad Warrana, Zone-B rocky and stony area of Tabbi Khawah to Speena Banda and ridge and mountain stretch of Zibi dim to Kanda Khel, Zone-C a hilly area of handia to the edge of mountain of kroza, Zone-D stretch mount of Bahadar Khell to the mountain edge of TERI and plains area of GURGURI to the plains and desert parts of Gurguri. During the survey of study Zone A-42, Zone B-53, Zone C-23, and Zone D-223 *Alectoris chukars* are seen. According to the obtained data, it was found that *chukar* mostly prefer insect then others items. During survey, majority of the respondents replied that there is illegal hunting, destruction of habitat, increasing of population, construction, environmental changes cause the decline population of *Chukar*. The areas, which *Alectoris chukar* occupied had dominant of *Acacia arabica*, *Acacia modesta*, *Dodnea viscosa*, *Dilbergia sisso*, *Pinus roxburghii* and *Pinus wallichiana*. There is an urgent need to conserve *Chukar* by improving their habitat in the study area, avoiding illegal hunting etc.

Keywords: *Alectoris chukar*, distribution, food Preference, threat

ANTIMICROBIAL RESISTANCE (AMR)

AR-1

Antimicrobial Photodynamic Therapy: A New approach for Antimicrobial Resistance

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ABSTRACT

Antimicrobial Photodynamic Therapy (aPDT) is based on application of three important parts including photosensitizer (PS), light at specific wavelength and oxygen which offers advantages over conventional antimicrobial treatments. In general, mechanism of aPDT action on microorganisms is light activated PS generates ROS, which mainly result in the killing of bacteria cells. PDT can use for treatment of different microorganisms such as bacteria, virus, and fungus with low side effects. The use of nano-vehicles is a promising strategy in potentiating aPDT by improving photosensitizer solubility, stability and targeting. Numerous studies are ongoing to determine the optimal combination approaches with other antimicrobial treatments. To conclude, more work is required for improvement of aPDT to bring it into clinics.

Keywords: Antimicrobial Photodynamic Therapy, Photosensitizer, Bacterial resistance, Combination Therapy Nanoparticle

Distribution and Frequency of *Tem* and *CTX-M* Genes in *Esbl* Producing *Enterobacter Cloacae*

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ABSTRACT

Enterobacter is a naturally occurring facultative anaerobic Gram-negative bacteria belonging to the *Enterobacteriaceae* family. These bacteria are saprophytic in nature and reside in soil and sewage. In recent decades, *Enterobacter* has gained clinical significance as a leading nosocomial infection-causing agent in critical care unit patients. This problem has been exacerbated by accumulating evidence of *Enterobacter cloacae*'s resistance to routinely prescribed antibiotics, particularly those causing extended-spectrum beta-lactamases (ESBL). Given this situation, it is crucial to regularly monitor antibiotic resistance levels and the frequency of causative genes in a population. Therefore, the current research focuses on determining the frequency of *TEM* and *CTX-M* genes in *E. cloacae* isolated from clinical samples. A total of 150 clinical samples (30 urines, 30 pus, 30 sputum, 30 blood, and 30 wounds) were collected from patients admitted or visiting Khyber Teaching Hospital in Peshawar to isolate ESBL-producing *E. cloacae*. Antibiotic resistance analysis of positive samples revealed the highest resistance to *Aztreonam* followed by *Ceftazidime*, *Ciprofloxacin*, *Piperacillin*, *Imipenem*, *Gentamycin* and *Cefepime*, *Amikacin*, *Meropenem*, and *Ertapenem*. Colistin, on the other hand, showed the least resistance. Polymerase Chain Reaction (PCR)-based analysis of *ESBL* genes revealed that the majority (47%) of resistant isolates had the *TEM* gene, while 38% had the *CTX-M* gene, and the remaining 15% were negative for both. In conclusion, *ESBL*-based antibiotic resistance is on the rise (1/3 of the samples tested positive), mainly caused (85%) by the presence of either the *TEM* or *CTX-M* genes. Medical practitioners should consider this alarming rise in resistance when prescribing antibiotics.

Keywords: Antibiotic resistance, *Enterobacteriaceae*, ESBL, *TEM*, *CTX-M*

Gender Wise Distribution of Tuberculosis and Pattern of Drug Susceptibility for Anti-TB Drugs in Khyber Pakhtunkhwa

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ABSTRACT

Tuberculosis (TB) is a disease of public health concern, responsible for approximately 10 million new infections and 1.5 million deaths in 2018. The aim of this study is to determine the prevalence of tuberculosis and drug resistance (including multidrug resistance) and to investigate possible relationships between drug resistance and gender. This study is designed to include a total of 4018 suspects of tuberculosis who visit Provincial TB Reference Laboratory at HMC Peshawar. The patients were screened for both pulmonary (PTB) and extrapulmonary tuberculosis (EPTB) using Fluorescence microscopy, Gene Xpert and culturing on both liquid and solid media. The positive samples were further processed for drug susceptibility pattern. In 4018 samples, 1855 were females and 2163 males. The prevalence of PTB in males was 240 (5.9%) and 210 (5.2%) in females. The EPTB was found higher in males 24 (0.59%) and 21 (0.5%) in females. In case of first line drugs, Isoniazid was the most resistant in females 50 (62.5%) and Ethambutol 23 (28.7%) the most effective drug. Similarly, in males, Isoniazid was the most resistant (29 (41.42%)) and ethambutol the most sensitive 64 (92.75%) observed. In case of 2nd line drugs, Levofloxacin was the most resistant drug in males and females with 34 (42.5%) and 26 (37.14%) respectively. The most susceptible drug was Amikacin in both genders. The cases of multidrug resistance were more in females 46 (57.5%) as compared to males 26 (37.14%). In this study, we found that tuberculosis is more common in males as compared to females. The pattern of drug susceptibility pattern is same in both genders. But however, MDR was more common in females.

Keywords: Tuberculosis, TB, males, females, drug susceptibility pattern, prevalence

Impact of Different Diagnostic Techniques on Timely Initiation of Treatment among Multidrug Resistant TB Patients

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ABSTRACT

Among patients with multidrug resistant- tuberculosis (MDR-TB), delays in diagnosis and treatment initiation are frequently observed, resulting in an increased risk of disease complications, extended period of TB infectivity, higher morbidity and mortality. The aim of the study was to find the effects of different diagnostic techniques on timely initiation of treatment among MDR-TB patients and drug-resistance against 1st and 2nd Line Anti-TB drugs. A cohort analysis was done using data on MDR-TB patients diagnosed during March 2022 February 2023 under PMDT, LRHPeshawar. Different diagnostic techniques including DST, LPA and Gene-Xpert MTB/XDR were used for detection of MDR-TB in pulmonary and extra-pulmonary samples. The patients were divided into three groups based on technique used i.e., Pre-LPA or DST, Post-LPA and Ten color Xpert MTB/XDR and followed up for their culture conversion. In this study, 180 positive MDR-TB patients were included. Gene-Xpert MTB/RIF showed 93% cases were positive and on DST all cases showed positive results. For Drug-resistance in DST, LPA and Gene-Xpert MTB/XDR, 61.1% patients were resistant to three first-line drugs and 11.1% resistant to second line. Overall median time from identification of patients suspected for MDR-TB to the initiation of treatment in DST was 93 days. In LPA it was 25 and 5 days by Ten-color Gene Xpert MTB/XDR. Mortality rate was lesser in LPA (2%) than DST (3%) and none in Ten-color Gene Xpert MTB/XDR. This study concludes that based on timely initiation of treatment and culture conversion reports, DST and LPA are less efficient than Ten-color Gene Xpert MTB/XDR assay.

Keywords: TB, MDR-TB, LPA, DST, Ten-color Gene Xpert MTB/XDR

Microbial Communities in Organ Infections: Biofilm and Antimicrobial Resistance Associations

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ABSTRACT

Antimicrobial resistance (AMR) has emerged as a pressing and urgent threat to public health, posing significant challenges to the effective prevention and treatment of persistent diseases. Despite various measures implemented in recent decades to address this issue, the global trends of AMR persist unabated, showing no signs of slowing down. Biofilms play a crucial role in the development of antimicrobial resistance. A biofilm is a structured community of microorganisms that is attached to a surface and embedded in a self-produced matrix of extracellular polymeric substances. These substances can include polysaccharides, proteins, and nucleic acids, creating a protective environment for the microorganisms within the biofilm. The current study was designed to assess the possible changes in the bacterial communities of different types of infection. Therefore the samples were collected and pooled from different locations of organs G1 (lower leg), G2 (Foot), G3 (Upper leg), G4 (chest) and G5 (carther). The total DNA of the swab samples were extracted and subjected to 16S DNA sequencing and metagenomic analysis. Additionally, the study sought to compare these findings with antimicrobial resistance (AMR) profiles, shedding light on potential correlations between biofilm-forming capabilities and resistance to antimicrobial agents within the microbial populations. Metagenomic analysis showed variations in relative abundance of 5 phyla, 17 families, 27 genera 9 classes, 12 order, and 38 species of bacteria between the different organs. The study identified diverse bacterial communities across various organ infections, revealing significant variations in biofilm-related microbial composition and providing insights into the correlation between these patterns and antimicrobial resistance profiles.

Keywords: Antimicrobial resistance, biofilm, metagenome, microbial composition

Detection of Extended Spectrum Beta Lactamase among *Escherichia coli* Isolates from Urinary Tract Infected Patients in Quetta, Pakistan

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ABSTRACT

Extended spectrum beta lactamases (ESBL) are enzymes produced by different species of Enterobacteriaceae family and are able to hydrolyze beta lactam ring. This study was designed to evaluate the ESBL producing *Escherichia coli* in urinary tract infected patients. A total of 100 urine samples were collected from patients with urinary tract infection in periurban area of Quetta. *E. coli* was confirmed as metallic sheen growth on Eosine methylene blue agar followed by IMViC test confirmation and reconfirmed through polymerase chain reaction through UidA gene amplification. Phenotypic confirmatory disc diffusion test and double disc synergy test were performed to detect the production of ESBL in confirmed isolates of *E. coli*. Results indicate confirmation of 39 *E. coli* isolates with 17/39 (44%) as ESBL producers through phenotypic confirmatory disc diffusion test and 13/39 (33%) by double disc synergy test. Moreover, *CTX-M* and *SHV* gene were detected in all 13/39 (33%) ESBL isolates. Present study indicates high frequency of resistance against *E. coli* infection with ESBL producers in the UTI patients of the target area. It is recommended to discourage self-medication and over the counter medicine to curtail the antimicrobial resistance in population.

Keywords: *Escherichia coli*, Urinary Tract Infections (UTI), Extended Spectrum Beta-Lactamases (ESBL), Antimicrobial Resistance, Phenotype, Genotype, Detection

Antibiotic Susceptibility Pattern of *Escherichia coli* Isolates from Clinical Specimens at PIMS, Islamabad

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ABSTRACT

Escherichia coli (*E. coli*) is the most common producer of extended spectrum beta lactamase enzyme (ESBL) which confers broad spectrum resistance to antibiotics like penicillin, cephalosporin and monobactam. The present study was carried out at Pakistan Institute of Medical Sciences, Islamabad. The marked resistance was viewed against amoxicilline-clavulanic acid, ceftriaxone and ceftazidime. The most effective drugs established were sulbactum-cefoparazone, amikacin, pepircillin-tazobactum. A total of 220 samples of wide range were selected, i.e., blood, urine, pus, sputum, etc. and were analyzed using various techniques of Gram staining and biochemical identification. After performing antibiotic sensitivity tests, 83% samples came out to be ESBL positive and 17% were ESBL negative. It was concluded that to ensure adequate treatment of infections arising especially from urinary pathogens and controlling spread of bacterial resistant strains, the continuous monitoring by bacterial susceptibility testing is essential.

Keywords: Susceptibility pattern, *Escherichia coli*, PIMS, amoxicilline-clavulanic acid, ceftriaxone, ceftazidime, sulbactum-cefoparazone, amikacin, pepircillin-tazobactum

Isolation, Characterization, Antimicrobial Activities and Antioxidant Potential of Endophytic Bacteria Isolated from *Moringa oleifera* Seeds

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ABSTRACT

Moringa oleifera is also known as horseradish tree that belong to family *Moringaceae*. Endophytic bacteria live inside plant host cell by producing secondary metabolites. The aim of this study was to isolate and characterize endophytic bacteria from *Moringa oleifera* seeds followed by investigating their plant growth promoting abilities and antibacterial role. *Moringa* seeds were surface sterilized with 0.5% mercuric chloride and cultured in LB agar media for the isolation of endophytic bacteria. Morphological analysis of the isolated endophytes was carried out using Gram staining procedure which confirmed the presence of single round shape gram negative bacteria. Molecular characterization of the endophytes was performed by extracting the DNA followed by amplifying it with universal 16S primers. The 16S RNA amplification revealed that the band size of the amplified target was around 720bp. The antibacterial activity of the endophytic bacteria was carried by culturing in broth media for 24 h at 25°C. The supernatant of the media was collected through centrifugation and was freeze-dried using lyophilizer. The powder obtained was used to determine its role against three different human pathogenic bacterial strains. The anti-oxidant potential of the extract will be performed via DPPH free radical scavenging activity. The results showed that endophytic bacterial extract was able to inhibit the growth of human pathogen, *Staphylococcus aureus* (3.6mm ± 0.13mm), *Klebsiella pneumonia* (4.3mm ± 0.21mm), and *Escherichia coli* (4.2mm ± 0.13mm) zone of inhibition.

Keywords: *Moringa*, horseradish, endophytic bacteria, antibacterial activity, anti-oxidant activity, *S. aureus*, *K. pneumonia*, *E. coli*

Antimicrobial Resistance Pattern and Detection of Resistance Genes in *Staphylococcus aureus* Isolated from the Raw Milk of Cows with Subclinical Mastitis

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ABSTRACT

Bovine mastitis is a widespread and costly disease that affects dairy farming globally, characterized by mammary gland inflammation. Bovine intramammary gland infection has been associated with more than 135 different pathogens of which *Staphylococcus aureus*, is the main etiology of sub-clinical mastitis (SCM). The current study aimed to determine the *S. aureus* prevalence in subclinical bovine mastitis milk samples, assess their resistance to commonly used antibiotics, and examine the *mecA*, *tetK*, and *vanA* resistant genes in *S. aureus* isolated strains. Out of 543 cow raw milk samples that were tested, 310 (57.09%) were positive for SCM. Among the SCM-positive samples, *S. aureus* was detected in 30.32% (94/310) samples. The *S. aureus* was confirmed through gram staining, coagulase and catalase tests. All the biochemically confirmed *S. aureus* isolates were molecularly identified using the thermonuclease (*nuc*) gene. *S. aureus* isolates were tested with the 10 empirically used antibiotics, 50% (47/94) isolates were determined to be multi-drug resistant (MDR). The *S. aureus* isolates showed the highest resistance to Lincomycin (84.04%) followed by Vancomycin (67.02%) and Ampicillin (45.74%) while the least resistance was showed to Sulfamethoxazole/Trimethoprim (3.19%) and Gentamycin (6.38%). PCR analysis showed that 46.80% of the MDR isolates harbored the *tetK* gene, while the *mecA* gene was found in 17.02% of the isolates, and none of the isolates carried the *vanA* gene. The present study infers that 50% of the *S. aureus* strains are multidrug resistant that carries a great public health threat. It is concluded that Sulfamethoxazole/Trimethoprim and Gentamycin were the most effective drugs tested against *S. aureus* in vitro.

Keywords: Bovine Mastitis, *Staphylococcus aureus*, Multidrug Resistance (MDR), Antibiotic Resistance Genes, Subclinical Mastitis

Detection of Multi Drug Resistant *Staphylococcus Epidermidis* Isolated From Raw Milk Samples of Dairy Cattle and Ewes

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ABSTRACT

The main objectives of this study were two fold: first, to assess the prevalence of subclinical mastitis and identify instances of multi-drug resistance (MDR) *S. epidermidis* in raw milk samples from dairy cattle and ewes; second, to explore the existence of antibiotic resistance genes. (*mecA*, *tetK*, and *ermC*) in the isolated *S. epidermidis* strains. During the study, 310 milk samples were collected, with 160 from cattle and 150 from ewes. The study revealed the overall 26% prevalence of subclinical mastitis. Within species, ewes had a slightly higher prevalence (31.34%) compared to cattle (21.87%). Among the breeds studied, Cholistani breed exhibited the highest prevalence of subclinical mastitis at 40%, whereas the Holstein breed had a prevalence of 14.3%. A total of 12.90% of milk samples (40 out of 310) were positive for *S. epidermidis*. Among these, 27.5% were isolated from healthy milk samples, and 72.5% were from infected milk samples. The *S. epidermidis* isolates were tested against 8 antibiotics, showing high resistance to Penicillin and Erythromycin (100%), followed by Cotrimoxazole (27.5%), Doxycycline (25%), Clindamycin (17.5%), and Chloramphenicol (15%). Levofloxacin and Ciprofloxacin had lower resistance rates (5%). The 27.5% of the isolates were multidrug resistant based on antimicrobial susceptibility testing. Regarding resistant genes, *ermC* was the most prevalent (87.5%), followed by *tetK* (80%), while *mecA* showed a lower prevalence (45%) among the *S. epidermidis* isolates. The research highlights the crucial role of antibiotic stewardship, which entails the responsible and prudent administration of antibiotics, as a vital strategy to effectively counteract the emergence of multidrug-resistant strains.

Keywords: Subclinical Mastitis, *Staphylococcus epidermidis*, Antibiotic Resistance Genes, Multidrug Resistance (MDR), Dairy Cattle, Ewes

Antimicrobial Activity of Medicinal Plant Extracts against Bacterial Strain Causing Gastrointestinal Diseases

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ABSTRACT

Herbal medicine derived from therapeutic plants is most abundant. Secondary bioactive compounds are responsible for the majority of the plant's therapeutic benefits. The abundance of phytochemicals found in medicinal plants allows for the structural optimization and synthesis of novel medications. Understanding the framework for the design and development of synthetic drugs has been made possible by medicinal plants. Furthermore, the development of therapeutic plants will impact the field of medicine because of the introduction of novel pathogens and diseases that require complementary or alternative therapies. The main objective of this research is to find out the antibacterial activity of crude extract. In this study plant crude extracts were checked against MDR GI pathogens by well diffusion method. Plant crude extracts exhibited best results against selected MDR GI pathogens. Further bioactive molecules isolation will be needed for therapeutic activity in pharmaceutical industry against MDR pathogens.

Keywords: Crude extract, Anti MDR activity. GI(Gastro intestinal diseases).

Distribution and Frequency of *TEM* and *CTX-M* Genes in ESBL Producing *Enterobacter cloacae*

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ABSTRACT

Enterobacter is a naturally occurring facultative anaerobic Gram-negative bacteria belonging to the *Enterobacteriaceae* family. These bacteria are saprophytic in nature and reside in soil and sewage. In recent decades, *Enterobacter* has gained clinical significance as a leading nosocomial infection-causing agent in critical care unit patients. This problem has been exacerbated by accumulating evidence of *Enterobacter cloacae*'s resistance to routinely prescribed antibiotics, particularly those causing extended-spectrum beta-lactamases (ESBL). Given this situation, it is crucial to regularly monitor antibiotic resistance levels and the frequency of causative genes in a population. Therefore, the current research focuses on determining the frequency of *TEM* and *CTX-M* genes in *E. cloacae* isolated from clinical samples. A total of 150 clinical samples (30 urines, 30 pus, 30 sputum, 30 blood, and 30 wounds) were collected from patients admitted or visiting Khyber Teaching Hospital in Peshawar to isolate ESBL-producing *E. cloacae*. Antibiotic resistance analysis of positive samples revealed the highest resistance to *Aztreonam* followed by *Ceftazidime*, *Ciprofloxacin*, *Piperacillin*, *Imipenem*, *Gentamycin* and *Cefepime*, *Amikacin*, *Meropenem*, and *Ertapenem*. Colistin, on the other hand, showed the least resistance. Polymerase Chain Reaction (PCR)-based analysis of *ESBL* genes revealed that the majority (47%) of resistant isolates had the *TEM* gene, while 38% had the *CTX-M* gene, and the remaining 15% were negative for both. In conclusion, *ESBL*-based antibiotic resistance is on the rise (1/3 of the samples tested positive), mainly caused (85%) by the presence of either the *TEM* or *CTX-M* genes. Medical practitioners should consider this alarming rise in resistance when prescribing antibiotics.

Keywords: Antibiotic resistance, *Enterobacteriaceae*, ESBL, *TEM*, *CTX-M*

COMPARISON OF LINE PROBE ASSAY WITH LIQUID CULTURE FOR DETECTION OF MULTI-DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS*(MDR-TB)

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ABSTRACT

MDR-TB is a major health concern worldwide, causing death of approximately 1.4 million HIV people in 2021. Countries like China, India, Pakistan and other low-income states are the high endemic countries. The way to control and eliminate this infection is by its appropriate and prompt diagnosis. Several diagnostic procedures are available but for its control and elimination, we need rapid, more efficient and timely detection techniques that would be helpful in achieving our goals. A simple cross-sectional study was conducted at PMDT-LRH Peshawar between April 2022 to September 2022. A total of 303 sputum samples were collected from patients of both genders and all age groups suspected to have TB infection. The presence of MTB was confirmed by AFB microscopy followed by GeneXpert, LPA for confirmation of MDR-TB. Liquid culture DST was used as standard technique as well as comparison with molecular diagnostic assays. Liquid Culture MGIT 960 system was used as reference gold standard for comparison with GeneXpert and LPA. The sensitivity, specificity and predictive values were calculated and compared for each diagnostic test. The sensitivity, specificity, PPV and NPV (95%CI) calculated for LPA were 94.6%, 88.5%, 94.65 and 88.5% respectively whereas for GeneXpert MTB/RIF it was found to be 90.8%, 91.6%, 95% and 82% respectively. It is concluded from this study that LPA was found to be more valid, quick and less time consuming (approximately 2 hours) when compared to other diagnostic procedures.

Keywords: Mycobacterium tuberculosis, TB, Diagnosis, LPA, Liquid culture, GeneXpert.

Development of an Antimicrobial Resistance-Targeted *E. coli* Vaccine for Broilers in Pakistan

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ABSTRACT

The research endeavored to devise an *E. coli* vaccine specifically tailored for broiler chickens in Pakistan, aiming to combat antimicrobial resistance. Through a series of rigorous experimental phases, the development of this groundbreaking vaccine was meticulously pursued. The study encompassed a detailed understanding of the prevalent strains of *E. coli* in broiler populations across diverse regions of Pakistan. Initially, extensive sampling from various poultry farms and regions was conducted, collecting *E. coli* strains to identify the prevailing antimicrobial resistance profiles. These strains were systematically analyzed to pinpoint the most recurrent and resilient antimicrobial-resistant variations. Subsequently, the identified strains underwent extensive genetic and antigenic characterization to elucidate the key targets for an effective vaccine. Following this, a comprehensive vaccine formulation was meticulously designed, incorporating these identified targets. The vaccine's efficacy and safety were rigorously assessed through in vitro and in vivo experiments. The immunogenic response in broilers was monitored, ensuring both the vaccine's capacity to trigger an immune response and its safety for the birds. The research team systematically analyzed the vaccine's efficacy in mitigating the colonization and transmission of antimicrobial-resistant *E. coli* within the broiler population. Encouragingly, the vaccine exhibited promising outcomes, showing a notable reduction in antimicrobial-resistant *E. coli* prevalence among vaccinated broilers compared to unvaccinated counterparts. Furthermore, this innovative vaccine underwent stringent regulatory assessments and was in the process of obtaining necessary approvals for mass production and subsequent distribution within the poultry industry. The successful development of this tailored vaccine not only presents a viable solution to combat antimicrobial resistance in broilers but also holds potential implications for improving food safety and public health in Pakistan's poultry sector.

Keywords: Antimicrobial Resistance, *E. coli*, Vaccine Development, Broilers, Pakistan, Targeted Immunization

Molecular Identification of Metal-Resistant Enterobacteriaceae in *Silver Carp* from River Kabul Khyber Pakhtunkhwa

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ABSTRACT

Metal-resistant Enterobacteriaceae pose a significant threat to aquatic ecosystems and public health, particularly when associated with fishes consumed by humans. The bacterial family Enterobacteriaceae had variable resistance to heavy metals such as Cd, Cu, Co, Zn, and Pb. This study focuses on the molecular identification of metal-resistant Enterobacteriaceae in Silver Carp (*Hypophthalmichthys molitrix*) from the River Kabul in Khyber Pakhtunkhwa. Silver carp were collected from three different sites. Bacterial samples were isolated from silver carp's gills, intestines, and opercula regions. The species of Enterobacteriaceae were isolated by spread plate method and identified by gram staining and biochemical tests. The positive bacterial isolates were then checked for metal resistance against Zn, Cu, Ni, and Hg by wells methods. More than 30% of collected fish were positive for metal-resistant bacteria. The isolates revealed the highest prevalence of resistance against Hg, followed by Cu, Ni, and Zn. *Klebsiella* and *E. coli* (Enterobacteriaceae) were among the dominant species identified molecularly using Sanger sequencing of 16S rRNA. The study highlights the substantial presence of metal-resistant Enterobacteriaceae, notably *Klebsiella*, and *E. coli*, in Silver Carp from the River Kabul. The variability in resistance patterns against heavy metals emphasizes the adaptability of these bacteria to diverse environmental stressors. Given the identified contamination risk, it is imperative to implement robust monitoring and management strategies to mitigate the spread of metal-resistant Enterobacteriaceae in aquatic ecosystems. Further research should explore the sources of metal pollution in the River Kabul and assess its impact on microbial communities.

Keywords: Enterobacteriaceae, *Escherichia coli*, *E. coli*, Silver Carp, Metal-resistance, 16S RNA, *Klebsiella*, metal pollution

Prevalence and Antibiotics Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Selected Pure Breeds of Cattle with Subclinical Mastitis

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ABSTRACT

A total of 308 milk samples were collected from selected pure breeds of cattle across Pakistan. Approximately 15 mL of milk was obtained after discarding the initial 2-3 milk streaks. After collecting the milk sample, the tip of the container was immediately sealed and placed in an ice box (-4°C). To isolate *P. aeruginosa*, the suspicious samples were grown on MacConkey agar media. All the isolates were phenotypically and biochemically confirmed through various tests i.e. Gram staining, Catalase test. The prevalence ratio of *P. aeruginosa* across different breeds is recorded highest in Cholistani 12/61(19.6%) followed by Achai, 1/5 (20%), Red Sindhi, 8/104 (7.69%) and the lowest ratio was recorded in Holstein Friesian 2/40 (5%). There were no positive sample detected in Sahiwal breed. Furthermore, antibiotic sensitivity test was performed using the nine commonly used antibiotics in veterinary medicine namely, ampicillin, penicillin, vancomycin, ciprofloxacin, gentamicin CN10, amikacin, streptomycin, chloramphenicol, amoxicillin, and tetracycline. The results showed that 23 samples showed resistance to ampicillin (100%), amoxicillin (78.37%), streptomycin (67.65%), vancomycin (60%), tetracycline (45%), amikacin (39%), gentamicin (21.74%), ciprofloxacin (8.7%), and chloramphenicol (4.5%). All the isolates were studied for presence of *blaTEM* and *blaSHV* genes with base pair 516 and 392, respectively. In overall 23 samples 16 samples were positive for *blaTEM* gene, with *blaSHV* gene in none of the isolates.

Keywords: Subclinical mastitis, *Pseudomonas aeruginosa*, Antibiotic resistance, resistant genes

Molecular Assessment of Fluoroquinolones Resistant *gyrA* and *parC* genes in *E. coli* Strains Isolated from Urinary Tract Infected Patients at District Mardan

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ABSTRACT

Escherichia coli (*E. coli*) is a rod-shaped and Gram-negative prokaryote that usually resides in the large intestine and is evacuated via feces. The urinary tract is the main site of infection of *E. coli*, and more than 90% of all primary urinary tract infections are caused by this organism. The objective of the study is to verify the overall relation of different generations of fluoroquinolone on *E. coli* genes (*gyrA* and *parC* gene) with respect to sensitivity, resistance, and pattern of infection. In this study, a total of 150 specimens were examined for urinary treatment infection (UTI); via microscopy and urine culture, 119 specimens were found UTI-positive. The specimens were also confirmed by biochemical tests. The antibiogram testing was performed for the identification of fluoroquinolone resistance ability. From 119 specimens, 84 isolates were found resistant to fluoroquinolone, with resistance percent around 85.3, 87.6, 81.4, and 77.1% for generations I, II, III, and IV, respectively. Further polymerase chain reaction (PCR) and agarose gel electrophoresis analysis (GEA) were performed. Through PCR, we came to know that, from a total of 84 resistant isolates, 29 (34.5%) and 41 (48.8%) isolates were resistant to *gyrA* and *parC* gene, respectively. The remaining 14 isolates showed resistance to both *gyrA* and *parC* (16.6 %). It is concluded from this study that *E. coli* is the primary source of urinary tract infections, and fluoroquinolones are no longer the drug of choice for its treatments in patients in the vicinity of the Mardan district since among its serotypes, about 85% are fluoroquinolone-resistant, and only 29.4% are sensitive. The *parC* is a more prevalent gene compared to *gyrA* in fluoroquinolone-resistant cases. In a few samples, both *gyrA* and *parC*-resistant serotypes were found simultaneously.

Key points: urinary tract infection, UTI, *E. coli*, fluoroquinolone, polymerase chain reaction, genes, *gyrA*, *parC*, gel electrophoresis.

Antibiotic Resistance Pattern of *Escherichia coli* Isolated From Raw Milk of Cow and Buffalo with Subclinical Mastitis

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ABSTRACT

Escherichia coli (*E. coli*), a Gram-negative, rod-shaped bacterium belonging to the Enterobacteriaceae family, is a common pathogen responsible for subclinical mastitis in dairy cattle. The overuse of antibiotics in mastitis treatment, lacking a comprehensive understanding, contributes to the emergence of multi-drug resistant strains, posing threats to both animal and human health. This study aimed to assess the prevalence of *E. coli* in subclinical mastitis, analyze its resistance patterns to various antibiotics, and investigate the presence of resistance genes (*TEM* and *CTX-M*) in *E. coli* isolates. A total of 310 milk samples were collected from lactating cattle and buffalo on dairy farms, comprising 160 samples from Cholistani and Holstein cattle breeds and 150 from buffaloes. The study revealed an overall subclinical mastitis prevalence of 34%, with buffaloes exhibiting a slightly higher prevalence (28.06%) than cattle (21.87%). Within cattle breeds, Cholistani breed showed a higher prevalence (40%) compared to Holstein breed (14.3%). *E. coli* was isolated from 14.51% of the total milk samples. Antimicrobial susceptibility testing (AST) of the isolated *E. coli* demonstrated the highest resistance to Chloramphenicol (100%) and the lowest resistance to Vancomycin (8%). Analysis of antimicrobial resistance genes revealed a higher prevalence of the *blaTEM* gene (55.55%) compared to *blaCTXM* (22.23%). These findings highlight the significance of understanding and addressing subclinical mastitis, particularly caused by *E. coli*, in dairy cattle. The observed resistance patterns and gene prevalence highlight the importance of judicious antibiotic use to mitigate the development of resistant strains. The implications of this research extend to the improvement of dairy industry practices and public health outcomes. The knowledge gained will aid dairy farmers in implementing effective mastitis management strategies, contributing to overall industry and public health enhancement.

Keywords: Antibiotic resistance, *Escherichia coli*, resistance genes, sub-clinical mastitis, somatic cell count.

Prevalence and Antibiotics Resistant Pattern of *Escherichia coli* Isolated from Pure Breeds of Cattle with Subclinical Mastitis

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ABSTRACT

Mastitis is an redness of the cow mammary gland that affects the choice and amount of milk. Streptococci, Staphylococcus, and *Escherichia coli* are the most often associated bacteria. *E. coli* is a common pathogen causing mastitis. The extent of *E. coli* caused bovine mastitis The study's goal was to investigate the incidence of *Escherichia coli* in preclinical bovine mastitis milk samples taken from reputable dairy cow farms. To determine the resistance pattern of *Escherichia coli* aureus isolates to routinely used antibiotics and to investigate the blaTEM and blaCTXM resistance genes in these isolates. In the current investigation, 305 milk samples were taken from seven different dairy cattle farms in Pakestan, with the frequency of *Escherichia coli* being 10.3% (n=32/305). Culturing on EMB media indicated the presence of *Escherichia coli*. Out of 32 verified Ecoli samples tested with the 9 empherically used antibiotics, 7 samples were determined to be MDR. The *Escherichia coli* isolates showed highest resistance to Chloramphenicol (100%) followed by Ampicilline (59.2%) and Amikacin (25%) while the least resistance was shown to Amoxicilline (9%) and Gentamycin (9.3%). Through PCR blaTEM, and blaCTXM resistant genes were checked in all 32 *Escherichia coli* MDR and AMR isolates. It was discovered that blaTEM 96% (n=31) MDR isolates harbored gene, was found in 90% blaCTXM(n=29). Antibiotic resistance in bacteria is exacerbated by misuse and ill-treatment of antibiotics in veterinarian field procedures. Reported to the study, farmers should only use antibiotics that bacteria are sensitive to, and antibiotic position is critical in bidding to reduce antimicrobial resistance.

Keywords: *Escherichia coli*, antibiotic resistant, resistant genes, mastitis

Prevalence and Antimicrobial Resistance of *Klebsiella pneumoniae* Isolated from Subclinical Bovine Mastitis in Selected Pure Dairy Cattle Breeds

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ABSTRACT

The aim of this study was to determine the prevalence and antimicrobial resistance genes *bla*_{TEM}, *bla*_{SHV} and *ermC* amongst *K. pneumoniae* isolated from milk samples obtained from pure cattle breeds of government dairy farms in Pakistan. In this study 305, milk samples were collected from various pure lactating breeds of different dairy farms including Red Sindi (n=104), Sahiwal (n=100), Holstein Friesian (n=40), and Cholistani (n=61). In the present study the rate of SCM was 56.39%; whereas the pervasiveness of *K. pneumoniae* was recorded as 11.8% in this study. The highest breed wise prevalence of *K. pneumoniae* was recorded in Cholistani 26.2% followed by Red Sindi 17.30% and lowest prevalence was recorded in Holstein Friesian 5%. The farm wise prevalence of *K. pneumoniae* was recorded higher in Govt. Livestock Farm Jugaitpeer Cholistani 26.2%, followed by Red Sindi Gharo Farm 21.21%, Red Sindi Cattle Farm Hub 13.04%, while the less prevalence was detected in Livestock Experimental Station Korangi Karachi 6.66% and Government Cattle Breeding and Dairy Farm Harichand 5%. Antibiotics susceptibility tests were performed which, revealed that overall 36 isolate were resistant to Ampicillin (100%). While highly effective sensitive antibiotic was Ciprofloxacin to which all the phenotypes shown 0% resistance. Isolates were found sensitive to Gentamycin 94.44% followed by Vancomycin 86.11%. PCR was used to examine antibiotic resistance genes in extended spectrum beta(β)-lactamase (ESBL) (*bla*_{TEM}, *bla*_{SHV}) and erythromycin (*ermC*) gene. In overall 36 samples 33 samples were detected positive for *bla*_{TEM} gene and seven samples were detected positive for *ermC* gene. The prevalence of *bla*_{TEM} and *ermC* was recorded 91.6% and 19.4%, while the *bla*_{SHV} gene was not reported. These findings indicated that ESBL producing *K. pneumoniae* isolated from SCM show a great public health concern.

Keywords: *Klebsiella pneumoniae*, Mastitis, Resistant genes

Therapeutic Potency of Selected Plants and Chromatographic Fractions against Multidrug-Resistant Bacteria Responsible for Human Dermatological Infections

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ABSTARCT

Medicinal plants have long played a pivotal role in traditional herbal remedies, holding a unique and esteemed position in the tapestry of global indigenous medicine systems. Recognizing their historical significance, this study delves into the phytochemical screening, antibacterial activity, and High Pressure Liquid Chromatography (HPLC) fractions of select indigenous plants. The focus is on their potential effectiveness against skin Multidrug-Resistant Bacteria (MDR). Local plant specimens were gathered and utilized for the extraction process. The resulting plant extracts and their HPLC fractions were then evaluated for antibacterial activity against skin MDR bacteria using the well diffusion method. Notably, the crude aqueous extract from these plants exhibited remarkable efficacy, displaying the highest inhibition zones against both *S. aureus* and *P. aeruginosa*. Furthermore, all HPLC fractions derived from the plant extracts demonstrated significant activity against skin MDR bacteria. This study contributes valuable insights into the bioactive potential of specific plants, emphasizing their role in combating skin Multidrug-Resistant Bacteria. The findings hold promise for the pharmaceutical sector in the pursuit of developing therapeutic interventions against MDR strains.

Keywords: phytochemical screening, antibacterial activity, HPLC fractions, MDR, *S. aureus*, *P. aeruginosa*.

Molecular Epidemiology of Antibiotic Susceptibility of Pathogenic Bacteria and Antimicrobial Resistance Genes of *Escherichia coli* Isolated from Urinary Tract Infection Patients

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ABSTRACT

To examine the antibiotic patterns of pathogenic bacteria and the presence of antibiotic-resistance genes in *E. coli* isolated from urinary tract infection patients. The study was conducted from March to August 2022 at the Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan. The study was designed to isolate and characterize pathogenic bacteria from UTI patients, as well as to type antibiotic resistance genes (*Mcr-2*, *Mcr-3*, *Bla-TEM*, *AAC(3) IV*, *Bla-SHV*, *Bla-CTX-M*, *aadA*, *astA*, *fana*, *estB*, *estA*, and *elt*) of *E. coli* strains. *E. coli* strains: 75% resistant to *Norfloxacin*, multidrug resistant (MDR) with resistance to a minimum of three antimicrobials. The predominant virulence gene, *estB*, exhibits a prevalence of 96.6% and is detected more frequently in female UTI patients (99.4%) compared to males (89.4%), showing a significant difference (*P-value*: 0.0001); both *astA* and *estB* genes are present in 99.4% of cases, with some isolates carrying 1 to 4 virulence genes. Strong correlation of antibiotic-resistance genes between female and male UTI patients observed. The study offers valuable insights, aiding clinicians and patients in effective treatment to reduce mortality rates globally, and in developing strategies for antibiotic sensitivity testing and therapeutics against UTI-causing pathogens.

Keywords: Urinary Tract Infection, UTI, *Escherichia coli*, *E. coli*, genotyping, microbial drug resistance, epidemiology

Antimicrobial Usage and Detection of Multidrug-Resistant *Staphylococcus aureus*: Methicillin- and Tetracycline-Resistant Strains in Raw Milk of Lactating Dairy Cattle

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ABSTRACT

The main objectives of this study were to determine the antimicrobial resistance pattern of *S. aureus* in raw milk and to detect the presence of *mecA* and *tetK* genes in it. A total of 150 milk samples were obtained aseptically from lactating cattle, including Holstein Friesian, Achai, and Jersey breeds, maintained at different dairy farms. The milk samples were checked for the presence of *S. aureus*, and it was detected in 55 (37%) of them. The presence of *S. aureus* was verified by culturing on selective media, gram staining, and performing coagulase and catalase tests. Further confirmation was performed through PCR with a species-specific thermonuclease (*nuc*) gene. Antimicrobial susceptibility testing of the confirmed *S. aureus* was then determined by using the Kirby–Bauer disc diffusion technique. Out of the 55 confirmed *S. aureus* isolates, 11 were determined to be multidrug-resistant (MDR). The highest resistance was found to penicillin (100%) and oxacillin (100%), followed by tetracycline (72.72%), amikacin (27.27%), sulfamethoxazole/trimethoprim (18.18%), tobramycin (18.18%), and gentamycin (9.09%). Amoxicillin and ciprofloxacin were found to be susceptible (100%). Out of 11 MDR *S. aureus* isolates, the methicillin resistance gene (*mecA*) was detected in 9 isolates, while the tetracycline resistance gene (*tetK*) was found in 7 isolates. The presence of these methicillin- and tetracycline-resistant strains in raw milk poses a major risk to public health, as they can cause food poisoning outbreaks that can spread rapidly through populations. Our study concludes that out of nine empirically used antibiotics, amoxicillin, ciprofloxacin, and gentamicin were highly effective against *S. aureus* compared to penicillin, oxacillin, and tetracycline.

Keywords: antimicrobial resistance, *Staphylococcus aureus*, mastitis, resistance genes, tetracycline, methicillin

Prevalence and Antibiotics Resistance Pattern of *Escherichia coli* Isolated from Lactating Dairy Breeds of Cattle in Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

The present study was designed to estimate the prevalence of *Escherichia coli* (*E. coli*) in the milk samples from sub-clinical mastitis, to determine the resistance pattern of *E. coli* isolates to empirically used antibiotics and to analyze TEM and CTX-M resistance genes in the *E. coli* isolates. This study was conducted in different dairy cattle breeds maintained at government dairy farms in Khyber Pakhtunkhwa, Pakistan. Out of total 150 isolates, 18 (12%) were positive for *E. coli* that were phenotypically and biochemically confirmed. Antibiotic sensitivity test revealed that (61.11%) were multidrug resistant. All of the 18 isolates were resistant to Augmentin (100%), followed by Ceftriaxone, Sulfamethoxazole-trimethoprim and tetracycline (55.5%) each. The most effective drug was Gentamycin to which resistance was 0%. Screening all the 18 positive samples through PCR revealed that eleven (61 %) of the total isolates had CTXM and seven isolates (39 %) had TEM resistant gene. Sub-clinical mastitis was more prevalent in the exotic breeds (Holstein Friesian and Jersey) was due to their huge milk production and their well-developed udder. Higher resistance was shown against beta lactam antibiotics. Due to prolonged and excess use of this antibiotic bacteria develops beta lactamases to overcome the effect of the drug.

Keywords: *Escherichia coli*, subclinical mastitis, antibiotic resistance, resistant genes

Prevalence, Antibiotics Resistance Pattern and Molecular Characteristics of *Staphylococcus Epidermidis* Isolated From Milk of Pure Breeds of Pakistani Dairy Cattle with Subclinical Mastitis

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ABSTRACT

This study set out to comprehensively investigate the landscape of *S. epidermidis* in dairy cattle afflicted with subclinical mastitis. We examined the prevalence of *S. epidermidis*, assessed its antibiotic resistance patterns, and probed for the presence of antibiotic-resistant genes (specifically, *MecA*, *TetK*, and *ErmC*) within *S. epidermidis* strains isolated from 305 milk samples across four distinct dairy cattle breeds: Holstein Friesian, Red Sindhi, Sahiwal, and Cholistani. Among the sampled cows, a staggering 56.39% (172/305) were found to be grappling with subclinical mastitis. Within this afflicted population, the prevalence of *S. epidermidis* reached 27.90% (48/172). Our breed-specific analysis revealed significant disparities, with Red Sindhi cows displaying the highest prevalence at 75%, followed by Holstein Friesian at 45.45%, and significantly lower levels in Sahiwal (5.19%) and Cholistani (3.44%) breeds. To assess the efficacy of antibiotics, we conducted sensitivity testing using nine commonly prescribed antibiotics. Alarmingly, 18 out of the 48 isolates (37.5%) exhibited multidrug resistance (MDR). Our antibiogram results underscored a high resistance of *S. epidermidis* isolates, particularly against Cefoxitin (56.25%) and Penicillin (43.75%), while demonstrating remarkable susceptibility to Amikacin (2.08%), Clindamycin (0%), Ciprofloxacin (0%), and Chloramphenicol (0%). Furthermore, we employed PCR to ascertain the presence of resistant genes in all *S. epidermidis* isolates. *MecA* was detected in 38 isolates (79.16%), while *TetK* was identified in 33 isolates (68.75%). Notably, the study did not detect the presence of the *ErmC* gene. Our investigation underscores the remarkable efficacy of chloramphenicol, clindamycin, and ciprofloxacin against *S. epidermidis*. However, the prevalence of multidrug-resistant *S. epidermidis* among mastitis cases highlights the urgent need for a more judicious and vigilant approach to antibiotic usage in veterinary practices.

Keywords: *Staphylococcus epidermidis*, subclinical mastitis, antibiotic resistance, resistant genes

Molecular Identification of Metal-Resistant Enterobacteriaceae in *Silver carp* from River Kabul Khyber Pakhtunkhwa

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ABSTRACT

Metal-resistant Enterobacteriaceae pose a significant threat to aquatic ecosystems and public health, particularly when associated with fishes consumed by humans. The bacterial family Enterobacteriaceae had variable resistance to heavy metals such as Cd, Cu, Co, Zn, and Pb. This study focuses on the molecular identification of metal-resistant Enterobacteriaceae in Silver Carp (*Hypophthalmichthys molitrix*) from the River Kabul in Khyber Pakhtunkhwa. Silver carp were collected from three different sites. Bacterial samples were isolated from silver carp's gills, intestines, and opercula regions. The species of Enterobacteriaceae were isolated by spread plate method and identified by gram staining and biochemical tests. The positive bacterial isolates were then checked for metal resistance against Zn, Cu, Ni, and Hg by wells methods. More than 30% of collected fish were positive for metal-resistant bacteria. The isolates revealed the highest prevalence of resistance against Hg, followed by Cu, Ni, and Zn. *Klebsiella* and *E. coli* (Enterobacteriaceae) were among the dominant species identified molecularly using Sanger sequencing of 16S rRNA. The study highlights the substantial presence of metal-resistant Enterobacteriaceae, notably *Klebsiella*, and *E. coli*, in Silver Carp from the River Kabul. The variability in resistance patterns against heavy metals emphasizes the adaptability of these bacteria to diverse environmental stressors. Given the identified contamination risk, it is imperative to implement robust monitoring and management strategies to mitigate the spread of metal-resistant Enterobacteriaceae in aquatic ecosystems. Further research should explore the sources of metal pollution in the River Kabul and assess its impact on microbial communities.

Keywords: Enterobacteriaceae, Escherichia coli, Metal-resistance, 16S RNA, *Klebsiella*

LIVESTOCK IMPROVEMENT (LI)

LI-1

Effects of Babesiosis on Blood Hematological and Biochemical Parameters of Cattle

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ABSTRACT

Babesiosis is a life-threatening disease caused by a parasite of the *Babesia* spp., which infects and destroys the red blood cells. The disease is transmitted mainly by ticks which become infected by feeding on infected cattle. The animals will face certain death if the disease is not properly getting treatment on time. Due to this disease the livestock industry suffers many problems and has faced with many challenges and losses. The objective of the current study is to find out the effect of hematological and biochemical parameters of babesiosis on cow blood. The present was carried out in summer season of Jalalabad, Nangarhar province in 2021. Total of 30 cows of Watani and 30 cows of Crossbred were selected. The cows were then divided by 15 babesia infected and 15 healthy cows as controlled group for both breeds. The sample for blood was collected from jugular vein of animals using EDTA tube which contains 2cc of blood for biochemical tests. The results showed that hematological parameters as RBCs and Hb in Watani and Crossbred cows were significantly decreased ($P \leq 0.05$) in babesia infected cows than healthy controlled group. While the amount of WBCs was significantly higher ($P \leq 0.05$) in babesia infected cows. In case of biochemical parameters, the amounts of AST and ALT both in Watani and Crossbred cows were significantly higher ($P \leq 0.05$) in babesia- infected cows. This studied concluded that the amount of total protein was significantly decreased ($p \leq 0.05$). The amount of cholesterol remained significantly higher. Both hematological and biochemical parameters were significantly different in babesia infected cows than controlled healthy cows in the present study.

Keywords: Babesiosis, cows, hematology, biochemical factors

Annotation of Potential Vaccine Targets and Designing of Mrna-Based Multi-Epitope Vaccine against Lumpy Skin Disease Virus via Reverse Vaccinology and Agent-Based Modeling

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ABSTRACT

Lumpy skin disease is a fatal emerging disease of cattle, which has started to gain extensive attention due to its rapid incursions across the globe. The disease epidemic causes economic loss and cattle morbidity. Currently, there are no specific treatments and safe vaccines against the lumpy skin disease virus (LSDV) to halt the spread of the disease. The current study uses genome-scan vaccinomics analyses to prioritize promiscuous vaccine candidate proteins of the LSDV. These proteins were subjected to top-ranked B- and T-cell epitope prediction based on their antigenicity, allergenicity, and toxicity values. The shortlisted epitopes were connected using appropriate linkers and adjuvant sequences to design multi-epitope vaccine constructs. Three vaccine constructs were prioritized based on their immunological and physicochemical properties. The model constructs were back-translated to nucleotide sequences and codons were optimized. The Kozak sequence with a start codon along with MITD, tPA, Goblin 50, 30 UTRs, and a poly(A) tail sequences were added to design a stable and highly immunogenic mRNA vaccine. Molecular docking followed by MD simulation analysis predicted significant binding affinity and stability of LSDV-V2 construct within bovine immune receptors and predicted it to be the top-ranked candidate to stimulate the humeral and cellular immunogenic responses. Furthermore, in silico restriction cloning predicted feasible gene expression of the LSDV-V2 construct in a bacterial expression vector. It could prove worth while to validate the predicted vaccine models experimentally and clinically against LSDV.

Keywords: lumpy skin disease virus; reverse vaccinology; vaccine designing; molecular modeling

Preparation of Frozen Yogurt as Healthier Option

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ABSTRACT

Yogurt has very high demand and consumption as dairy item. With the advancement of technology the product should be improved. Frozen yogurt is relatively a new concept and it has many benefits, having low calories and fat content, high calcium and potassium (USDA Food Composition Database). Frozen yogurt is also rich in gut friendly bacteria (Pei et al., 2017), which are helpful in good gut health and aids digestion. Frozen yogurt is also a treat for lactose intolerant people as it has low level of lactose (Elli et al., 2006). This project will help to provide a healthier option as frozen desert to the consumers.

Keywords: frozen yogurt, probiotics, gut health, lactose intolerance, nutritional benefits

Evaluation of Physical Properties of Saliva as Non-Invasive and Point-Of-Care Diagnostic Tools for Early Pregnancy Detection in Cow

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ABSTRACT

The present study aims to develop a cost-effective pregnancy detection model by combining early pregnancy detecting parameters. It changes the saliva's physical properties in response to early pregnancy in cattle, validates these changes as a diagnostic tool, and develops a point-of-care testing system. The study analyzed the various physical properties of saliva, including pH, buffering capacity, crystallization, density, flow rate, and electrical conductivity. The study was conducted at a different government cattle breeding and dairy farm in District Charsadda and Mardan. The total number of animals were 200 (100 pregnant and 100 non-pregnant). The samples were processed in the Physiology laboratory of CVS & AH, AWKUM. The results showed that mean pH in pregnant cows was significantly higher ($P \leq 0.05$) (9.325 ± 0.13) than nonpregnant (8.133 ± 0.13). The mean Specific gravity (0.000173 ± 0.00), and Conductivity (0.666 ± 0.029) in non-pregnant were higher ($P \leq 0.05$) than the pregnant cows (0.000146 ± 0.00) and (0.538 ± 0.028). The mean buffer capacity (7.40 ± 0.10) and flow rate (91.92 ± 1.13) non-pregnant were higher ($P \leq 0.05$) than in the pregnant animals (2.42 ± 0.151) and (91.92 ± 1.13). The mean density (0.172 ± 0.03) non-pregnant were higher ($P \leq 0.05$) than the pregnant animals (0.172 ± 0.03). T. In pregnant cows, Fern-like patterns are 26.19, fir-like 4.76, and Branch-like 11.90, Dot-like 9.52, Branche-fir 7.14, Branche-fir-fern 4.76, branch-fern 19.04, fern-fir 7.14, none 9.52. In non-pregnant cows, fern-like 0.00, fir-like 6.45, bran-like 0.00, Dot-like 19.35, branch-fir 29.03, branch-fern-fir 19.35, branch-fern 22.58, fern-fir 0.00, none 3.22. The study found that pregnant animals had higher pH values, less flow rate, and buffering capacity, and decreased specific gravity and conductivity of saliva compared to non-pregnant animals. The results of the study suggested that the changes in saliva physical properties can be used to detect the early pregnancy by using non invasive techniques.

Keyword: Biomarker, non-invasive, pregnancy, physical properties, saliva.

Association of SNPs in *CD4* Gene with Mastitis and Milk Production Traits in Different Pure Dairy Cattle Breeds in Pakistan

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ABSTRACT

Mastitis is a chronic, inflammation of the mammary gland of cattle caused by bacteriological infections. It is a life-threatening disease that contributes to several diseases in dairy cattle, resulting in massive economic losses throughout the world. Keeping this in view, worldwide dairy cattle breeding programs are trying to breed cows with better resistance to mastitis. *CD4* gene play a crucial role in immune mechanisms against a variety of inflammatory diseases. The current study investigates the effects of single nucleotide polymorphisms (SNP) in the *CD4* gene on mastitis to open new doors for improving milk production and cattle health. 302 lactating dairy cow maintained at government dairy farms within Pakistan, were used to collect Blood and Milk samples from two different breed (Holstein Frisian and Cholistani). SNP were genotype using chine cow SNP chip. selected DNA samples followed by genotyping of those SNPs in all samples and statistical analysis by the overall linear model of SAS (9.4). One SNP of *CD4* gene SNP: rs110774456 T>C, Chromosome 5/104010752 were found associated significantly with the annual milk yield (AMY, Protein Percentage (PP) and Somatic Cell Score (SCS) (AMY, PP, SCS) $p < 0.05$. Our study suggest that SNP SNP: rs110774456 T>C of *CD4* showing resistance against mastitis will be recommended to be used in the breeding programs to improve the udder health and reduce the incidence of mastitis. This will help to increase the economic benefits of local farmers and the dairy industry of the country.

Keywords: Mastitis, *CD4* Gene, Single Nucleotide Polymorphisms (SNPs), Dairy Cattle Breeding, Milk Production, Somatic Cell Score (SCS), Annual Milk Yield (AMY), Protein Percentage, Genetic Resistance

Incidence of Gastrointestinal Parasites Found in Large Ruminants in District Lower Dir Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Large ruminants are the major source of milk and milk product as well source of proteins which play an important role in the economy of Pakistan. The present study was aimed to investigate the prevalence of gastrointestinal parasites among large ruminants in Lower Dir of Pakistan. Randomly fecal samples obtained directly from the rectum of Buffalos and Cows using gloved fingers and put in clean sampling plastic bottles containing 10% formalin solution and recorded the socio-demographic data in questioner asked from the owner. The overall prevalence of gastrointestinal parasites was found 58.59% (184/314) followed by Cow 63.55% (75/118) and buffalo 55.61% (109/196) respectively with the occurrence of *Entiamebia* 17.43% (19/109), *Moniezia* 24.77% (27/109), *Haemunchus* 40.36% (44/109) and *Coccidia* 17.43% (19/109) in cows while the prevalence of *Entiamebia* 25.33% (19/75), *Moniezia* 14.66% (11/75), *Haemunchus* 53.33% (40/75) and *Coccidia* 6.66% (5/75) in buffalos were found. Young age Buffaloes and Cows found more prevalent 73.8% (62/84) and 56.12% (22/41) respectively. Grazing cows 76.19% (64/84) and stall feed Buffaloes 65.75% (48/73), Cows drinking open water 71.11% (32/45) and Buffaloes 67.53% (52/77), Cows and Buffaloes in summer 68.8% (86/125) and 71.64% (48/67) found infected. It was concluded that Cows of study area were highly affected by gastrointestinal parasites especially *Haemunchus* which need special attention for control and screening.

Keywords: prevalence, cows, buffaloes, gastrointestinal parasites, *Haemunchus*, Lower Dir.

Spatiotemporal Patterns, Risk Factors and Molecular Characterization of Ascarid Nematodes in Cattle Population

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ABSTRACT

Ascarid nematodes are parasitic worms that infect various parts of host including cattle and cause significant economic and health concerns. The present study aimed to explore the spatiotemporal patterns, risk factors and provide molecular data of ascarid nematodes in cattle. 337 faecal samples were collected in six different study areas from cattle to examine larvae, adult worms or eggs. The diagnosis of ascaridiosis were usually made by routine fecal examination and genetic approach. DNA was extracted from the worms using a DNA extraction and purification reagent kit. The ITS-1 and ITS-2 region were amplified by polymerase chain reaction (PCR) and sequence were compared with NCBI (GenBank) database using Staden Package. A phylogenetic tree was constructed by (NJ) method. Out of 337 samples, 278 were found positive for Ascarid parasites. Overall parasitism 83.38% (281/337) with 89.63% (173/193) in grazing cattle and 75% (108/144) in stall-fed cattle. The prevalence in poor body condition cattle were 42.5%, while the prevalence were 37.9% in healthy body cattle. The analysis of the sequencing involved the ITS-2 region 500 bp was found to be 100 percent identical to previously published *Taxocara vitulorum* ITS-2 sequence at NCBI (Y09492). And ITS-1 amplicons 300 bp were sequenced and found to be 99.3 percent identical to previously available *T. vitulorum* ITS-1 sequence at NCBI (AJ007455). The phylogenetic tree was constructed with Bootstrap support (Bp) values for the nodes that was 90% and it shows 100% homology with *T. vitulorum*. This study revealed a higher prevalence of Ascarid nematodes especially *T. vitulorum* in poor body cattle grazing in open area compared to healthy body cattle that were stall-fed more susceptible to infection.

Keywords: Ascaridiosis, *Taxocara*. Cattle, Genetic, Risk factors.

Genetic Polymorphisms in *JAK2* Gene are Associated with Mastitis Resistance and Milk Productions Traits in Different Pure Dairy Cattle Breeds of Pakistan

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ABSTRACT

The present study was designed to investigate the effects of single nucleotide polymorphisms (SNPs) in the *JAK2* gene on the milk production and mastitis related traits in dairy cattle. Blood and milk samples were collected from 406 lactating dairy cattle of four breeds, i.e. Holstein Friesian (HF), Achai (A), Red Sindhi (RS) and Sahiwal (S) maintained at well-established dairy farms in across Pakistan. Three SNPs, i.e. SNP 1 (T>C, rs210148032), SNP 2 (T>C,rs135128681), and SNP 3 (T>C, rs377935625) in *JAK2* were screened through Chinese Cow's SNPs Chip-I (CCSC-I) and genotyped in a population of 406 dairy cattle. The results were analyzed using the general linear model in SAS 9.4. Of the three SNPs, SNP 3 did not obey Hardy-Weinberg equilibrium ($P < 0.01$), while SNP 1 and SNP 2 were found to be in strong linkage disequilibrium and allele T was highly prevalent compared to allele C in these SNPs. In SNP 1, the TC genotype was associated with significantly ($P < 0.01$) higher annual milk yield (AMY). whereas in SNP 2, the CC genotype was significantly ($P < 0.01$) associated with higher lactose percentage (LP) compared to the other genotypes. SNP 3 was found significantly associated with the frequency of mastitis (FOM), fat percentage (FP), protein percentage (PP) and LP ($p < 0.05$). Altogether, our findings suggested that the SNPs of *JAK2* gene could be useful genetic markers in selection for milk production improvement and mastitis resistance phenotypic traits in dairy cattle.

Keywords: *JAK2* Gene, SNPs, Milk Production Traits, Mastitis, Dairy Cattle Breeding, Holstein Friesian, Achai Cattle, Red Sindhi, Sahiwal, Genetic Markers, Milk Yield

Molecular Characterization and Associated Risk Factors of *Theileria annulata* in Small and Large Ruminants of District Karak

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ABSTRACT

Ectoparasites, particularly hard ticks (Ixodids), pose a substantial threat to global livestock economies, causing significant economic losses and transmitting various diseases. Theileriosis, caused by different species of *Theileria*, notably *Theileria annulata* (*T. annulata*) and *Theileria parva*, is a prevalent hemoparasitic disease affecting numerous animal species. The aim of this study was molecular detection of *T. annulata* and to identify its associated risk factors in small and large ruminants of district Karak, Pakistan. A total of 157 blood samples were collected from designated areas and morphologically examined for *Theileria* presence via microscopic analysis. DNA extracted from positive samples underwent PCR using the *Tams-1* genetic marker to detect *T. annulata* infection in both small and large ruminants. Microscopic examination revealed a overall 21.01% (33/157) prevalence of theileriosis, with a higher prevalence among large ruminants 69.6% (23/33) compared to small ruminants 30.3% (10/33). PCR analysis determined an overall relative prevalence of 64% (11/17) in large ruminants and 35% (6/17) in small ruminants for *T. annulata*. Risk factor analysis highlighted significant associations with age, seasons, tick infestation, breed, and acaricide application, while gender did not demonstrate significance. The current study underscores the prevalence of *T. annulata* in ruminants in the Karak district and elucidates key factors contributing to its spread. These findings underscore the importance of comprehensive control measures to mitigate the impact of this parasitic infection on livestock health and productivity.

Keywords: *Theileria annulata*, *Tams-1* marker, PCR, small ruminants, tick infestation, Epidemiology

Molecular Detection and Risk Factors Analysis of Bovine Cryptosporidiosis in Diarrheic Calves in District Mardan, Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Diarrhea throughout the world there is considerable medical and veterinary significance of this enteric protozoan parasite that affects both humans and animals throughout the world. In developing countries, it is a major human and livestock health concern. The purpose of the current study was to determine the prevalence of *Cryptosporidium* infection in bovines through a demographic and clinical perspective. A total of 384 samples were collected from diarrheal and semisolid cows located in various areas of Mardan. The prevalence rate of the disease was calculated by taking into account different risk factors (age, season, humidity, and clinical symptoms). In order to stain the samples, the MZN staining technique was applied, followed by polymerase chain reaction. Staining techniques revealed that 92 out of 384 samples were positive. Furthermore, a polymerase chain reaction was performed on the positive samples. 60 out of 92 were found *C. Parvum* positive. The prevalence of *Cryptosporidium* was higher in district Mardan, with large number of risk factors. In order to determine the prevalence of cryptosporidiosis in the area and to identify possible risk factors associated with its occurrence, further research is needed.

Keyword: Calves, Cryptosporidiosis, Molecular Detection, PCR, Prevalence

Burden of Theileriosis Using Microscopy and PCR and its Impact on Hematological Profile of Infected Bovines in District Swat

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ABSTRACT

Bovine Theileriosis is an important tick-borne disease infecting bovines throughout the world, including Pakistan, resulting in big economic losses to the livestock industry, which contributes significantly to the country's GDP. Data regarding the prevalence of this disease in the region was not described. The present study aimed to determine the morphological and molecular prevalence of bovine Theileriosis, associated risk factors and to assess its effects on different hematological parameters of the infected bovines in District Swat, KP, Pakistan. Blood samples were collected from 306 animals (buffaloes and cattle) in the region, which were then diagnosed morphologically by microscopic examination and molecularly by polymerase chain reaction. The microscopic examination revealed an overall prevalence of 10.4% (35/306), while the PCR based prevalence was 18.9% (58/306). PCR based prevalence in cattle was 21.5% (37/172), while in buffaloes was 15.6% (21/134). The hematological analysis indicated that there are significant changes in different hematological parameters of the Theileria infected bovines. There was significant decrease observed in the red blood cells counts, hemoglobin, mean corpuscular hemoglobin concentration, hematocrit value platelets and white blood cells, while significant increase was observed in the lymphocyte percentage, mean corpuscular volume and mean corpuscular hemoglobin of the infected animals. Whereas other hematological parameters remain unaffected in the infected bovines. There was significant association of the prevalence of the disease with different characteristics of the host animal. Highest prevalence was found in Ticks infested (41/123, 33.33%), full grazing (19/51, 37.2%), female (38/184, 20.7%), exotic breeds (13/50, 26%), cross breeds (34/147, 24.3%) and animals having no acaricides treatment (42/120, 35%). Seasonal patterns were also associated with the rate of the infection. The rate of infection in summer was 23.9% (47/197), while in winter was 10.1% (11/109). The risk factors identified in this study should be considered while designing control measures to reduce the prevalence of the Theileriosis. More studies should be conducted to find the prevalence and diversity of Theileria species in the region.

Keywords: Theileriosis, Bovine, Hematology, Swat

Epidemiological Investigation of Foot and Mouth Disease in Small Ruminants Border Area Pakistan with Afghanistan

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ABSTRACT

FMD is a serious disease that affects ruminants and causes huge economic losses worldwide. In Pakistan, the impact of FMDV on small ruminants has not been well studied. We conducted a Sero epidemiological study in small ruminants near the Pakistan-Afghanistan border to estimate the prevalence of FMD in sheep and goats. We collected 800 serum samples from sheep (n = 424) and goats (n = 376) and tested them for antibodies against SP and NSP of FMD virus using enzyme-linked immunosorbent assay. We found that 42.5% (340/800) of the samples were positive for NSP, and that the most common FMD serotypes were O (44.5%), Asia-1 (42%) and A (32%). Sheep (39%; 95% CI: 34-44) had a lower (P<0.05) prevalence of FMD than goats (46%; 95% CI: 41-51). We also observed significant (P<0.05) differences in the seroprevalence of FMD-SP and -NSP proteins among different areas of the study region. Several risk factors, such as age, sex, breed, season, flock size, body condition, animal movement and production system, were associated with the distribution of FMD virus. Our study revealed that FMD is highly prevalent in sheep and goats near the Pakistan-Afghanistan border. We recommend that border-level teams should be established to investigate FMD outbreaks and develop risk-based surveillance and control plans for small ruminants to protect the country's economy.

Keywords: FMD, small ruminants, seroprevalence, Pakistan-Afghanistan border, risk factors

Morphologically and Molecularly Prevalence of *Babesia ovis* and *Babesia motasi* in Small Ruminants and its Associated Risk factor in District Dir Lower

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ABSTRACT

Babesiosis is a tick-borne disease that infects small ruminants worldwide, including Pakistan, causing huge economic losses to the livestock industry, which contributes greatly to the country's GDP. The present study was designed to document the microscopic and molecular prevalence of *Babesia* species (*Babesia ovis* and *Babesia motasi*) and associated risk factors in small ruminants of District Dir Lower, KP Province, Pakistan. A total of 400 blood samples including 200 sheep and 200 goats were collected in the study area. The samples were collected from the animals with variable age, both sexes, tick infestation and healthy. The collected samples were screened through microscopy followed by molecular confirmation through PCR in the Pathology Laboratory of College of Veterinary Science and Animal Husbandry, AWKUM. The microscopic examination showed 10% (200) *Babesia Ovis* found in sheep. While 6% (200) *Babesia motasi* found in goats. Molecular based prevalence was 19% (76/400) found in sheep and goats respectively. The PCR based prevalence of *Babesia Ovis* was 23.5% found in sheep, while no *Babesia ovis* was found in goats. The molecular prevalence of *Babesia motasi* was 14.5% found in goats, while no *Babesia motasi* was detected in sheep. There was significant association of the prevalence of the disease (*Babesia ovis* and *Babesia motasi*) with different characteristics of the small ruminants. The highest prevalence 23% was observed in tick infested sheep and goats. The prevalence of *Babesia ovis* and *Babesia motasi* in full grazing animals (sheep and goat) was 50% (35/70) while semi grazing (sheep and goat) was 14.7% (28/190) and stall feeders (sheep and goat) was 9.2% (13/140). While the prevalence of *Babesia Ovis* and *Babesia Motasi* in sheep and goat having no acaricide treatment was 29% (54/186). The current study reflects that Babesiosis is a seasonal disease and the prevalence of *Babesia Ovis* and *Babesia motasi* in summer season was 29.1% (70/240) observed, while in winter it was 3.7% (6/160) respectively. The main factors that increased the prevalence of *Babesia Ovis* and *Babesia motasi* infection in small ruminants (sheep and goat), were breed of the animal, presence of ticks, gender, seasonal pattern, acaricide treatment, and feeding method.

Keyword: *Babesia ovis*, *Babesia motasi*, PCR, Risk factor, Dir Lower

Serological, Molecular Detection and Potential Risk Factors Associated with Brucellosis in Cattle and Human

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ABSTRACT

The goal of the current study was to find out the molecular prevalence of brucellosis in the cattle and human populations as well as to investigate possible risk factors for brucellosis in district swat. A total of 420 samples, 210 each from humans and cattle were randomly collected. Total 30 samples from humans and cattle were taken from each of the seven tehsils in the district Swat. Sex, age, breed type, and location were among the studied risk factors. All samples were first screened through ELISA and then subjected to PCR, targeting the BCSP31 gene in *brucella* DNA for comparing the sensitivity and specificity of two diagnostic techniques. On ELISA, the overall prevalence was found as 2.38% and 3.8% in humans and cattle, respectively. While the overall prevalence recorded by PCR in humans and cattle was 3.33% and 4.28% respectively. Among humans higher prevalence was found in female 3.80 % and 5.71% through ELISA and PCR, respectively. Furthermore, in cattle higher prevalence was found in female through ELISA and PCR as 6.66% and 7.61%, respectively. Through ELISA, the Achai breed of cattle had the highest prevalence of 5.71% followed by the cross and Holstein Friesian as 4.28% and 1.42%, respectively. The seropositivity in two distinct breeds and a cross bred cattle was also determined by PCR as 5.71%, 4.28%, and 2.85%, respectively. Age-specific prevalence showed that adults had a higher prevalence rate of 7.61% on both ELISA and PCR. This study revealed a substantial correlation between risk factors such as sex, age, breed type, and location with brucellosis disease.

Keywords: Brucellosis, Cattle, ELISA, PCR, Zoonotic disease

Prevalence of *Cryptosporidium* Infection in Dogs and Cats in District Mardan, Pakistan

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ABSTRACT

Cryptosporidium spp. is an important gastrointestinal protozoan parasite that causes diarrhea in humans, wild and domestic animals. Several waterborne and animal's origin outbreaks have been reported globally. Although its infection is still a major public health problem, there is still uncertainty about its dogs and cats epidemics, especially in developing countries. A questionnaire was created to collect the demographic and clinical data from the pet's owner. The prevalence of *Cryptosporidium spp.* oocysts in stool samples from dogs and cats in the area of district Mardan was investigated in this study. A total of 128 feces samples were collected, from dogs 86 and from cats 42 samples were stained using the modified Ziehl Neelsen's (mZN) staining technique. The actual prevalence rate of *Cryptosporidium* infection was 29% in dogs and 21% in cats. The positive and negative predictive values (PPV & NPV) of these techniques also suggests that mZN staining can be used for daily diagnosis due to its low cost, easy availability and less time consumption. The findings indicated that the prevalence of parasitism owing to *Cryptosporidium spp.* in dogs and cats was relevant within the population investigated, emphasizing the asymptomatic character of this infection. The need of implementing control measures is emphasized. The results further revealed that individuals having diarrhea were more likely to be infected with *Cryptosporidium* infection. The prevalence of *Cryptosporidium* infection was high in district Mardan that was studied. However, there was statistically a significant relationship between *Cryptosporidium* infection and the other environmental variables that were evaluated. While, there was statistically a significant association between *Cryptosporidium* infection and different environmental factors that were evaluated. The present high prevalence in the research area might be owing to a lack of adequate diagnosis and regular based testing. More studies is needed to determine the importance of *Cryptosporidium* infection in the area, as well as to find out the specific risk factors associated with it.

Keywords: *Cryptosporidium spp.*, prevalence, dogs, cats, Modified Ziehl Neelsen

Molecular Diversity Analysis and Pathogens' Survey of *Argas persicus* Ticks Infesting Hen from Pakistan

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ABSTRACT

Ticks pose significant health risks to both wildlife and humans due to their role as vectors for various pathogens. In this study, distribution and genetic relationships within the tick species *Argas persicus*, focusing on its prevalence in hens (*Gallus gallus domesticus*) across different districts in Pakistan. We examined 750 hens for a whole year and identified an overall mean intensity of 2.19 ticks per infested chicken and an overall mean abundance of 1.61 ticks per examined hen. All collected ticks (n=1210) were morphologically identified as *A. persicus*, including 719 males, 333 females, 121 nymphs, and 38 larvae. The highest tick prevalence was observed in the Kurram district, followed by Bagh and Muzaffarabad, with the lowest in Kotli. Molecular analysis was conducted on tick DNA, revealing genetic similarities among *A. persicus* ticks based on 16S rDNA and ITS2 sequences. The surveillance of vector-borne pathogen infections in *A. persicus* indicated the absence of infections by *Babesia*, *Rickettsia*, and *Anaplasma* in all examined samples. This absence of detectable infections highlights the unique pathogen profile in the studied *A. persicus* population, with no evidence of these specific vector-borne pathogens. Phylogenetic analyses based on mitochondrial 16S rDNA and 12S rDNA genes illustrated the genetic relationships of *A. persicus* ticks from Pakistan with other *Argas* and species, providing insights into their evolutionary history as well as their genetic diversity. These findings contribute to our understanding of tick infestation patterns, tick-borne pathogens profile, and genetic diversity in hens of the selected region. **Keywords:** *Argas persicus*, Molecular diversity, phylogenetic analysis, haplotype diversity, prevalence, Pakistan.

Molecular Detection and Risk Factors Analysis of *Cryptosporidium* from Different Sheep Herds Reared in Malakand Region, Pakistan

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ABSTRACT

In Pakistan, minimal studies have been reported on Cryptosporidiosis in animals and sheep, considered an essential reservoir of *Cryptosporidium*. This study was designated to determine the prevalence of *Cryptosporidium* infections in sheep herds of Pakistan and to evaluate the influence of potential risk factors such as age, seasons, animal sex, drinking sources, management of herds, breeds, and symptoms of the infection. Four hundred forty-eight samples from 26 sheep herds were screened microscopically and then confirmed through PCR amplification of the 18S SSU rRNA gene. Our result revealed that out of 448 samples, 107 were found positive by Microscopy and 77-positive by PCR, with prevalence rates of 23.88% and 17.18% in different breeds of sheep. There is a high statistical difference between the P. value ($P < 0.05$) When compared with diarrheic and non-diarrheic sheep, breeds, ages, genders, and seasons. The highest infection rate was found in the Australian breed (8.25%); season-wise, the highest prevalence was recorded in summer. Furthermore, sheep-secreting diarrhoeal faces were (41%). This study reveals that young sheep and lambs are more susceptible to infection than adults and cryptosporidial infection decreases with increasing age of the animal. Based on the present recorded data, the infection rate was high in the study area; further studies are needed to determine the importance of *Cryptosporidium* with various risk factors and molecular characterization for identifying exact species. Due to the potential zoonotic nature of the infection, serious monitoring is needed regarding animal handling, treatment, and management and environmental contaminations for local sheep forming to prevent humans and other livestock from this outbreak.

Keywords: *Cryptosporidium*, outbreak, Prevalence, Sheep, Zoonotic

Prevalence and Epidemiology of Different Ticks Infesting Livestock of Khyber Pakhtunkhwa

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ABSTRACT

Ticks (Acari: Ixodidae) are obligate ectoparasites of diverse hosts that affect livestock globally and are carriers of a number of bacterial, viral, and protozoan infections that affect both animals and people. Ticks (Class Arachnida) are ectoparasites of a wide variety of vertebrates, including cattle, livestock and wild animals. Ticks are arachnids of veterinary and medical importance due to the fact that they can transmit various diseases. Accurate identification of tick species is crucial for effective disease surveillance, prevention, and control strategies. Morphological identification, based on the examination of key external features, remains a fundamental and widely used approach for tick taxonomy. This study provides a comprehensive overview of the morphological characteristics used in the identification of ticks, focusing on the main genera and species of medical and veterinary significance. Animals weaken and perform significantly worse after a tick infection and subsequent blood sucking. In Pakistan, tick infestation is a major contributor to tick-worry, tick-borne illnesses in cattle, and related economical losses. However, the production of livestock is significantly hampered by the harm that parasites and illnesses transmitted by ticks produce. The cattle in Pakistan are subject to year-round ticks infection, but little is known about the epidemiology, variety, and prevalence of tick species throughout the country. The purpose of the current study was to demonstrate the epidemiological characteristics relating to the prevalence of ticks and associated risk factors of tick infestation and to look at the frequency and variety of hard ticks that infest goats in District Mardan, Buner and Dir of Khyber Pakhtunkhwa. Our studies shows tick diversity, infestation rate, and numerous factors (season, age, and gender of host) influencing tick infestation rate in different goats and sheep in District Mardan, Buner and Dir (lower) in Province Khyber Pakhtunkhwa. A large number of small ruminants were considered in these selected areas. Among the identified species *Haemaphysalis* and *Rhiphicephalus* was the most prevalent genera. To build a precise nationwide model of tick infestation, more study is required to confirm our findings in the area and to validate our findings in other locations of Pakistan. It is possible to boost domestic animal productivity while reducing the spread of deadly tick-borne illnesses in society by educating farmers about ticks and providing nearby veterinary services. A large scale study is needed to explore the hard ticks diversity across the country and awareness is needed to minimize the risk of infection, especially among farmers and farms owners.

Keywords: Ticks, Goats, Sheep, Tick Infection, Tick Prevalence, *Haemaphysalis*, *Rhiphicephalus*

NOVEL APPROACHES IN COMBATTING DISEASES

CD-1

Endoscopic Evaluation of Gastric Mucosa to the *H. Pylori* Infection Status in the Recent Advances

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ABSTRACT

H. pylori infection is known as a type 1 carcinogen of gastric carcinoma and early diagnosis and treatment is essential for eradication. Recent findings have also identified that advanced atrophy and intestinal metaplasia of gastric mucosa persist after successful eradication too, that increase the risk of gastric cancer at an intermediate level compared to those who have not been infected. So, assessment of gastric mucosa during gastroscopy is very useful. The aims of this review are to identify and collect the reliable literatures and suggested features on newly developed endoscopic studies and methods of *H. pylori* status and gastritis that influence it in clinical practice. Therefore, Conventional Endoscopic, Image Enhanced Endoscopic methods and Kyoto classification related studies are searched and reviewed. The conventional endoscopic Kyoto classification due to worldwide use, low-cost, and time-efficient with new findings is a good method for evaluation of *H. pylori* current, past, and no infection status. Advanced Image Enhanced Endoscopic methods such as NBI, LCI and BLI combine with Magnifying Endoscopy are provide a relatively cleared endoscopic features for *H. pylori* infection status and early gastric cancer. According to *H. pylori* infection status, endoscopic prediction of gastric mucosal surface architecture analysis is possible, which influences clinical management. These endoscopic techniques might lead us to easier diagnose and treatment of *H. pylori* infection and be useful not only in the eradication of *H. pylori* but also in the diagnosis of early gastric cancer.

Keywords: *Helicobacter pylori*, *H. pylori*, Gastric Carcinoma, Endoscopic Diagnosis, Kyoto Classification, Image-Enhanced Endoscopy (IEE), Early Gastric Cancer

Biocompatible Expression and Functional Characterization of a Cost-Effective DEFB-TP5 Peptide in *Pichia pastoris*: Implications for Anti-Inflammatory and Antimicrobial Applications

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ABSTRACT

DEFB-TP5 is a novel auspicious health-beneficial peptide derivative from two naturally occurring peptides, β -Defensin (DEFB) and thymopentin (TP5), and shows strong anti-inflammatory activity and binds to LPS without cytotoxicity and hemolytic effect. Furthermore, the application of DEFB-TP5 peptide is inadequate by its high cost. In the current study, we developed a biocompatible mechanism for expression of the DEFB-TP5 peptide in *Pichia pastoris*. The transgenic strain of hybrid DEFB-TP5 peptide with a molecular weight of 6.7kDa as predictable was obtained. The recombinant DEFB-TP5 peptide was purified by Ni-NTA chromatography, estimated 30.41 mg/L was obtained from the cell culture medium with 98.2% purity. Additionally, The purified DEFB-TP5 peptide significantly ($p < 0.05$) diminished the release of nitric oxide (NO), TNF- α , IL-6, IL-1 β in LPS-stimulated RAW264.7 macrophages in a dose-dependent manner. This study will not only help to understand the molecular mechanism of expression that can potentially be used to develop an anti-endotoxin peptide but also to serve as the basis for the development of antimicrobial and anti-inflammatory agents as well, which also provides a potential source for the production of recombinant bioactive DEFB-TP5 at the industrial level.

Keywords. Peptide, DEFB-TP5, chromatography, antimicrobial, inflammatory agents

Effects of *Moringa oleifera* Leaf Powder Supplements on Histomorphometry and Immune Responses of Broiler Chicken under Dexamethasone Induced Stress

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ABSTRACT

This study aimed to explore histomorphometry and immune responses of broilers to *Moringa oleifera* leaf powder (MOLP) supplements under dexamethasone (De) induced stress. Hundred-day-old chicks were divided into five groups having four replicates, (5 birds/replica). Group A (negative control) was fed basal diet (BD), B (positive control) with De (15mg/kg of diet after day 21st) + BD, C with De + 0.8% MOLP, D with De + 1.2% MOLP, and E with De + 1.6% MOLP. On 35th day, (2 birds/replica) were slaughtered for sampling (bursa of Fabricius (BF), cecal tonsils, intestine). Lymphatic follicular width and area of BF was higher ($P \leq 0.05$) in 1.6% MOLP group, lymphatic follicular number and length was non-significant in all groups. Lymphatic nodules number, length, width and area of cecal tonsils were non-significant in all groups. Antibody titers against Newcastle disease virus were unaffected and antibody titers against sheep red blood cells were significant ($P \geq 0.05$) in 1.6% MOLP treated birds on 21st and 35th day, cell-mediated immunity was non-significant. Also, after 72 hours, cell-mediated immunity was significant ($P \leq 0.05$) in 1.2% and 1.6% MOLP groups and acidic, mixed, and total goblet cells count were significant ($P \geq 0.05$) in all intestinal portions in MOLP groups except acidic goblet cells in duodenum. Number of Intra-epithelial lymphocytes in MOLP groups were not affected except ileum ($P \leq 0.05$). It was determined that 1.6% MOLP in broiler feed had a moderate effect on broiler's immunity.

Keywords: Broiler, dexamethasone, immunity, phytochemicals, stress

Identification and In-Silico Analysis of Non-Synonymous Mutations in *NOS3* Gene: A Comprehensive Study

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ABSTRACT

Endothelial NOS (eNOS), also known as nitric oxide synthase 3 (*NOS3*) or constitutive NOS (cNOS), is an enzyme that in humans is encoded by the *NOS3* gene located in the 7q35-7q36 region of chromosome 7. Nitric oxide synthase endothelial (*NOS3*) plays a crucial role in the production of nitric oxide, a signaling molecule that acts as an important endothelium-derived vasorelaxant factor. The disruption of *NOS3* or the inhibition of nitric oxide synthesis is significantly involved in a lot of diseases. Nonsynonymous Single nucleotide polymorphism (nsSNP) is involved in the pathogenesis of several diseases such as hypertension, preeclampsia, diabetes mellitus, obesity, erectile dysfunction, migraine, and cardiovascular diseases. To find out about Functional SNPs in such a disease-related gene on a large scale is very difficult so this study is conducted on a short population. This study aims to identify non-synonymous SNPs and their insilico analysis. The protein sequence of this gene is obtained from the ENSEMBLE database. Deleterious SNPs are identified by computational tools PANTHER, POLYPHEN, PHD-SNP, SIFT, and SNP AND GO. From this study, it is concluded that among 614 missense variants, only 40 are deleterious and affect protein function. Gene stability was analyzed by using I-Mutant and Mupro which showed that out of 40 nsSNPs, 34 showed a decrease in stability on I-MUTANT, and 37 decreased in stability on Mupro. A genetic mutation's possible effects can be predicted using an online server called Mutpred, which shows among 40 nsSNPs 38 are potentially dangerous. The 3D structure modeling of *NOS3* and 40 nsSNPs was also obtained from the I-TASSER tool. Gene mania was utilized to model the activity of *NOS3* with other genes and its significant results shows that *NOS3* physically interacted with *NOS1*, *NOS2*, *NOSIP*, *LYPLA1*, *NOSTRIN*, *GUCYA1A*, and *AKT1*. Protein-protein interaction of the *NOS3* gene was discovered through the STRING database which reveals 11 nodes and 49 edges. This study paves the way for further study on a large population and will also help in the diagnosis of diseases and the development of medicines for diseases that are associated with the *NOS3* gene.

Keywords: Endothelial Nitric Oxide Synthase (eNOS), Single Nucleotide Polymorphism (SNP), Nitric Oxide, *NOS3* gene, Protein Stability, In Silico Analysis, Cardiovascular Diseases

Identification and In-Silico Analysis of Non Synonymous Mutations in *AGT* Gene: A Comprehensive Study

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ABSTRACT

Angiotensinogen (*AGT*) is one of the most important gene in Renin-angiotensin-aldosterone system. This system is essential in the regulation of blood volume, electrolytes balance and systematic vascular resistance. *AGT* is the unique precursor of all angiotensin peptides. Non synonymous Single nucleotide polymorphism (nsSNP) in *AGT* gene have been associated with may disease including hypertension, cardio vascular diseases, ischemic stroke, obesity and diabetes mellitus. Functional SNPs identification in such type of disease associated gen uncomplicated so, for this reason before going for larger population, it's good to get information about putative functional SNPs. This study was carried out to identify non synonymous nsSNPs and its insilico analysis. Protein sequence was retrieved by using ENSEMBLE database. Identification of deleterious nsSNPs is carried by using computational tools PENTHER, POLY-PHEN, PhD-SNP PhD-SNP and SIFT. The study concluded that out of 475 missense variants 21 (R26W,R26Q,V27G,H30Y,H30P,H30R,P31L,L34R,L125P,L210Q,R228C,R228H,R228L,Y272N,Y272C,G277E,W313R,P338L,M372R,P450Q and P472Q) were non-tolerated by all tools and thus effect protein function. Gene stability analysis using I-Mutant and Mupro showed out of 21 nsSNPs, 19 nsSNPs of them have show decrease in stability. Evolutionary profile of each amino acids present in the *AGT* protein is studied by using ConSurf that shows 12 nsSNPs are Highly conserved and Functionally exposed, 3 were exposed 6 were buried and 4 were Highly conserved and buried. This study also proposed 3D- structure of *AGT* and its most damaging 21 nsSNPs variants along with post translational modification of *AGT* gene showing no methylation is occur while phosphorylation occur at 5 positions in which at 4 nsSNPs position threonine was phosphorylated and at 1 nsSNP it occur on serine. This study makes a ladder for large scale studies and also helpful in developing medicine for cure of disease in which *AGT* gene is involved.

Keywords: Angiotensinogen (*AGT*), Renin-Angiotensin-Aldosterone System (RAAS), Single Nucleotide Polymorphism (SNP), Protein Stability, Hypertension, In Silico Analysis

Synthesis of Metallic Nanoparticles Using *Citrus sinensis* and *Prunus Armeniaca* Extract and its Medicinal Uses

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ABSTRACT

Nanoparticles are viewed as fundamental building blocks of nanotechnology. The use of nanoparticles is gaining attention in the present century as they possess defined chemical, optical and mechanical properties. Herein, we reported a green approach for the synthesis of NiO-NPs using *Citrus sinensis* and *Prunus armeniaca* extracts which has several advantages over the conventional methods. The as synthesized NiO-NPs were confirmed with the help of UV-Vis spectroscopy, FT-IR, and SEM techniques. The SEM images showed that *C. sinensis* NiO-NPs were roughly spherical and rectangular type while *P. armeniaca* NiO-NPs were irregular in shape. Both the NiO-NPs showed good antibacterial activity against *E.coli* followed by other bacterial species. The *C. sinensis* NiO-NPs and *P. armeniaca* NiO-NPs showed good antioxidant activity with an IC₅₀ value of 143.30 and 184.61 µg/mL respectively at highest concentration of 1000 µg/mL. Similarly the *C. sinensis* NiO-NPs and *P. armeniaca* NiO-NPs showed significant inhibition of alpha amylase enzyme with an IC₅₀ value of 253.25 and 229.2 µg/mL respectively at 1000 µg/mL. The NiO-NPs were biocompatible with HRBCs at highest concentration. The overall results of this study suggest that the NiO-NPs of *C. sinensis* and *P. armeniaca* extracts could be used in various biomedical applications.

Keywords: Nanoparticles, Green synthesis, Biological applications.

Evaluation of Serum Electrolytes in Diabetes Mellitus in District Mardan

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ABSTRACT

This study aimed to evaluate basic electrolytes (Na⁺, K⁺, Cl⁻) in patients with diabetes mellitus and to find the association of electrolytes imbalance with social demographic characteristic of the participant in Mardan. Venous blood (5ml) will be taken from randomly selected people and random glucose level will be determined. Glucose level above 200 mg/dl in people will be considered diabetic where as low level will be considered non diabetic. A total of 600 people, 300 diabetic and 300 non diabetic. Serum will be separated for the blood of the participants and will be analyzed for serum electrolytes. Social-demographic characteristics of the said participants will be recorded by using questionnaire or face-to-face interviews. The questionnaire included questions about age, weight, BMI, blood pressure, education, occupation, marital status, smoking, and residence. Blood glucose levels will be measured using the enzymatic reaction method with architect, while serum electrolytes levels will be measured using easy electrolyte. The gathered data will be analyzed with SPSS version 16 by using the chi square test, with statistical significance set at P < 0.05. The overall prevalence of electrolyte imbalance was found to be more in diabetic as compare to non diabetic patients with electrolytes like sodium, potassium and chloride being biased towards patients such as Hyponatremia 39.66% (95%CI:34.12–45.2), hypokalemia 5.66% (95%CI:3.04–8.28) and hypochloremia 36.66% (95%CI:31.21–42.11). Illiterate were more likely to electrolytes imbalance compare to literate, obesity, married, elderly, blood pressure and urbanization showed significant association with electrolyte imbalance.

Keywords: Dsiabetes Mellitus, Electrolyte Imbalance, Sodium (Na⁺), Potassium (K⁺), Chloride (Cl⁻), Demographic Factors

Invitro Antagonistic Activities of Exopolysaccharides Producing Endophytic Bacteria Isolated from Seeds of *Moringa oleifera* against Phytopathogenic Fungi

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ABSTRACT

Endophytes are microorganisms that colonize the internal plant tissues without causing any adverse effects, Furthermore, endophytes provide atmospheric nitrogen fixation, growth promoting substances, stress tolerance and resistance to nematodes and pathogens. Over the last twenty years, biological control has become an important alternative to control pests and diseases that affect plants, especially with the emergence of pest resistance to chemical pesticides and for the protection of the environment and to ensure sustainable agriculture. Endophytic EPS are considered as promising candidate for pharmaceuticals, bioremediation and bioleaching industries. EPS play a key role in plant-endophyte relations and are crucial for their survival in the host plant. Endophytes are well known for the production of biologically active secondary metabolites. In the current study, EPS-producing bacterial strains were isolated from *Moringa Oleifera* and identified by 16s rDNA sequencing. EPS was purified from the isolate and characterized by biophysical techniques. EPS production was quantitatively measured by thin layer chromatography. Further, invitro antagonistic activities of EPS producing bacterial isolate was tested against two plant pathogenic fungal strain, *A.alternata* and *F.solanae*. A total of 8 bacterial strain were isolated from *Moringa* seeds and were given name serially (KS1-KS-8). Out of 8 bacterial endophytes 2 isolates were selected for secondary screening of eps production. The bacterial isolates KS-2 and KS-7 showed mucoid colony on nutrient agar plates. Amongst the two bacterial isolate, KS-7 produced 0.95 g/L eps while the isolate KS-2 produced 0.44 g/L exopolysaccharides. The bacterial isolate was tested for their antifungal activities by dual culture plate method. Fungal plug (0.7mm) of pathogenic fungus was placed in the center of 90mm PDA (potato dextrose agar) plate and the overnight culturing tested endophytic bacteria strain was spot inoculated 2.5 cm away from fungal plug, PDA plate with only fungal plug served as control. The plates were incubated at 28 °C for five days. The bacterial isolate KS-7 showed the antagonistic activities against two phytopathogenic fungi *A.alternata* and *F.solanae*. The isolate KS-7 inhibit 50.06 % growth of *A.alternata* while inhibit the growth of *F.solanae* 53.48 % as compared to control plate.

Keywords: Endophytic Bacteria, Exopolysaccharides (EPS), *Moringa oleifera*, Antifungal Activity, Phytopathogenic Fungi, *A. alternata*, *F. solanae*

NMR-Based Metabolic Fingerprinting of Grapevine Cultivars

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ABSTRACT

Metabolic fingerprinting is the metabolic comparison of different samples indicating either different stages of development, disease of healthy state, or taxonomical differences, etc. This tool is now widely applied to the agriculture to achieve various goals, including quality control, disease and pest management, and assistance to breeding or genetic modification programs. Chemical characterization of economically important crops, i.e., chemotaxonomy, is one of the major objectives accomplished by the metabolic fingerprinting studies. Not only different types and samples and crops are studied, analytical platforms used for such studies also varied considerably. Liquid and gas chromatography coupled with mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy are the most widely used. Nuclear magnetic resonance is an optimum choice offering discrete advantages of simple sample preparation, shorter measurement time, and the use of spectroscopic data for quantification without any calibration curve. Current study used NMR in combination with various multivariate data analysis methods to perform chemical classification or chemotaxonomy of resistant and susceptible cultivars. The differentiating metabolites are identified as phenylpropanoids and flavonoids, whose concentrations are higher in the resistant cultivars.

Keywords: Metabolic Fingerprinting, Chemotaxonomy, Nuclear Magnetic Resonance (NMR) Spectroscopy, Phenylpropanoids, Flavonoids, Resistant Cultivars

Larvicidal Assessment and Dose-Response Relationship of Pakistani Bee Propolis against Mosquito Larvae

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ABSTRACT

A resinous substance known as Propolis is collected by bees from the buds and exudates of plants and then combined with pollen, wax, and their enzymes. Mosquito-borne diseases are a major global health concern. The present study aimed to investigate the larvicidal potential of propolis from Pakistani bees to pursue sustainable and effective vector control. The study was carried out in the Entomology Laboratory Department of Zoology KUST, Kohat. The Sample was collected from stagnant water and identified under a stereo-zoom microscope. Propolis was crushed into smaller fragments. To dissolve the propolis, 25 grams of crushed propolis was allowed to soak in 500 milliliters of 99% ethanol in a conical flask. The extracts of propolis were evaluated through a series of controlled experiments, for their efficacy in inducing larval mortality at different concentrations. The study provides a thorough analysis of the dose-response relationship, offering insights into the optimal concentrations needed for the highest level of larvicidal activity. The results of the present study showed that the extracts of propolis have significant larvicidal activity against mosquito larvae, with mortality rates reaching up to 93.33% on 1mg/ml. Additionally, our findings revealed that larval mortality rates were highest at a concentration of 1mg/ml with a dosage of 200 ul when compared to the positive control Lambda Cyhalothrin. This positive control made it possible to compare the larvicidal effectiveness of propolis directly against a well-known commercial drug. To verify our findings, we also used ethanol as a solvent in a negative control group. The importance of the current study extends beyond its practical uses. Propolis made from Pakistani bee populations is a locally available and sustainable resource. The findings contribute to the growing body of knowledge about natural resource-based approaches to the management of vector-borne diseases and offer a viable path forward for the creation of eco-friendly larvicides. The recognition of the dose-response relation provides a key basis for the precise and accurate administration of propolis.

Keywords: Mosquito, Vector-borne disease, Propolis, Vector control, Larvicide, Dose-response relationship.

Prevalence and Treatment Outcomes of Atrial Fibrillation among Hypertensive Patients

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ABSTRACT

Atrial fibrillation (AF) is the most prevalent supraventricular arrhythmia characterized by chaotic contraction of the atrium. Among all causing factors hypertension is the most prevalent condition in AF patients. It is known to increase the risk of developing new onset AF by 1.8 folds and increase the risk of progression to permanent AF by 1.5 folds. The current study aims to determine the number of hypertensive patients presenting with AF, co-morbidity associated with it and the treatment outcomes of AF in hypertensive patients. Sample size was 216 patients. Location CCU KTH Peshawar. Study design Retrospective study. The record was analyzed manually and graphs were plotted using Microsoft excel 2015. Total number of hypertensive patients with atrial fibrillation visiting cardiology department in one year was 216. The other co-morbidities associated with atrial fibrillation along with hypertension was diabetes mellitus followed by coronary heart disease, congestive cardiac failure, ischemic heart disease, COPD and hyperthyroidism. The outcome of new onset AF with pharmacological cardioversion (81 patients) were quite high as compared to DC cardioversion (9 patients). Amiodarone was given to all patients for pharmacological cardioversion, which was quite successful in given study population. All patients presented with new onset AF were discharged with sinus rhythm. In patients with persisted AF presented with FVR, rate was normalized mostly with digoxin 81%. The outcome of persistent AF patients discharged with normal heart rate was 100%. For long term rate control, 49 (40%) patients had a good response to combination of digoxin and cardioselective beta-blockers. Total number of hypertensive patients presented with AF were 216 in one year, among these ratio of both sexes were same and the incident of new onset AF were low as compare to persistent AF. The outcome of new onset AF with pharmacological cardioversion is high as compared to DC cardioversion and all of them were discharged with sinus rhythm. Amiodarone showed a high ratio of successful cardioversion in our study population. In case of persistent AF rate control with the combination of digoxin and beta blockers was quite high.

Keywords: Atrial Fibrillation, Hypertension, Pharmacological Cardioversion, Amiodarone, Digoxin, Beta-Blockers

Phage Therapy: A Biological Approach for the Treatment of Infectious Diseases

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ABSTRACT

The emergence of phage therapy as a potential alternative treatment has drawn considerable attention in the ongoing battle against antibiotic-resistant bacteria and persistent infectious diseases. Bacteriophages or phages are naturally occurring viruses that selectively infect and eliminate particular types of bacteria, presenting a targeted and precise strategy for addressing diseases. The growing concern regarding antibiotic resistance has led to the need to investigate other approaches, and phage therapy emerges as a promising solution in this context. The objective of this study is to present a comprehensive analysis of phage therapy, encompassing its historical background, contemporary progress, and its potential to overcome the limitations associated with conventional antibiotic treatments. Phages provide a focused and flexible strategy that allows for precise targeting of bacterial infections, hence reducing the potential harm to the human microbiota. This study aims to investigate the mechanism of action exhibited by phages, focusing on their capacity to infect and lyse bacterial cells, ultimately resulting in the elimination of harmful pathogenic strains. Some hurdles encompass the limited ability of phages to infect a specific range of hosts and the imperative requirement for comprehensive characterization of phage preparations. Furthermore, this study aims to examine existing research and clinical trials that provide evidence of the effectiveness of phage therapy in the treatment of several bacterial infections, thereby highlighting its capacity to transform the field of medicine potentially. This will obviously foster further research, thus establishing a novel era in the management of infectious diseases.

Keywords: Antibiotic Resistance, Bacteriophages, Clinical Applications, Infectious Diseases, Phage Therapy.

Assessment of Lumpy Skin Disease Virus Prevalence and Molecular Characterization in Cattle from District Kohat, Based on the P32 Gene

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ABSTRACT

Between January 2022 and December 2022, Lumpy Skin Disease Virus (LSDV) 286 cases were registered in the district Kohat, Khyber Pakhtunkhwa. Lumpy Skin Disease Virus (LSDV) is a member of the family Poxviridae and the genus *Capripoxvirus* causes viral infection. Lumpy Skin Disease (LSD) is an infectious, eruptive, and occasionally causes deadly illness. The cattle sector suffered significant financial losses as a result of the pandemic. The National Veterinary Laboratory (NVL) in Islamabad did a retrospective analysis by evaluating preserved veterinary data from the LSDV outbreak. Over a one year research period, a total of 286 cases in which 162 deaths (56.6%) were observed, with males having the highest prevalence (74.4%) at age group 1- 3 years but females having the highest mortality (61.8%) due to inadequate immunity against LSDV infection and a lack of effective treatment or vaccine. Sur Gul (86.95%) and Shakardara (87.5%) areas having the greatest frequency and death rates of Lumpy skin disease was observed in district Kohat. The most recorded outbreaks occurred in July (154.5%) and May (100%), with the highest mortality rate finding in (Autumn) September (100%) and the lowest reported cases happening in December (42.8%). A total of 50 representative specimens (skin biopsies) were taken from infected cattle's nodular skin lesions, and the P32 gene was effectively amplified by PCR in 56% (28/50) of all tested tissues following viral DNA isolation. Twenty (71.4%) out of the 28 amplification positive samples were chosen for DNA sequencing and 20 sequences were submitted to GenBank with assigned accession numbers: OM793602.1-MW452620.1. Phylogenetic analyses of the 20 sequences was done by using MEGA 11 and showed that the viruses formed seven clusters implying that at least two strains of LSDV are in circulation in Kohat, Pakistan. Cluster V and VI Pakistan LSDV isolates 100 percent similarity with cluster VII due to both strains present in south Asia (Pakistan and India). Clusters III, IV Pakistan LSDV isolates 97 percent similarity with cluster I and II because reference strains were from south East Asia (Russia, Taiwan, China, and Thailand). The incidence and molecular characterization of LSDV in Kohat, Pakistan is first reported in this paper.

Keywords: Lumpy Skin Disease Virus, Capripoxvirus, Molecular Characterization, Phylogenetic Analysis, Cattle Mortality, Pakistan (Epidemiology)

Agaricus Subgenus Pseudochitonia with Description of three New Species from District Malakand, Pakistan

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ABSTRACT

Agaricus is a large genus containing more than 500 species of tropical or subtropical climate. District Malakand has also a subtropical climate and selected for the taxonomic study of the genus *Agaricus*. The present study deals to description of a total of nine species with three new species of *Agaricus* subgenus *Pseudochitonia*, as new record based on morphological characteristics and phylogenetic analyses using three DNA regions: nuc ribosomal DNA internal transcribed spacers (ITS), fragments of the large subunit of nuc ribosomal DNA (28S) and the translation elongation factor 1 alpha gene (*TEF1*). One new species viz *Agaricus lanosus* with wooly squamules on its cap, forms a lineage within *Agaricus* sect. *Bivelares* which cannot be classified with certainty in one of the two subsections (*Cupressorum* and *Hortenses*) of this section. *Agaricus rhizoideus* with rhizoid like structure at the base of the stipe, forms a basal clade in *Agaricus* sect. *Hondenses*. Specimens of the third new species, *Agaricus malakandensis* form a species-level clade within *Agaricus* sect. *Catenulati* and exhibits the morphological characteristics of this section. Due to their similar ITS sequences, two previously unnamed specimens from Thailand (*A. sp.* LD2012162 and CA799) are considered conspecific with *A. malakandensis*.

Keywords: *Agaricus*, *Pseudochitonia*, Malakand, DNA internal transcribed spacers, new species

Exploring Anti-Diabetic and Anti-Inflammatory Effects of Endophytic Fungi-Derived Exopolysaccharides from Wheat and Parthenium

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ABSTRACT

Endophytes are microbes that colonize living, internal tissues of plants without causing any negative effects. In recent years, endophytic fungi have been demonstrated to be excellent exopolysaccharides (EPS) producers. EPS are high-molecular-weight carbohydrates with a wide range of biological activities. This study was, therefore designed to extract and purify EPS-producing endophytic fungi from wheat (*Triticum aestivum*) and Parthenium (*Parthenium hysterophorus*) and explore their biological applications using different bioassays. The purified EPS were labelled as EPSW₁, EPSW₂, EPSW₃, EPSP₁, EPSP₂, and EPSP₃. The purification of EPS involved inoculating isolated endophytic fungi into a broth medium at 25°C for 32 hours on a shaking incubator and EPS precipitation using 95% ice-chilled ethanol. The purified EPS were processed for quantification of total carbohydrate content and bioactivity through α -amylase, α -glucosidase and HBRCs membrane stabilization assay. The results indicated that EPSW₁ inhibited α -amylase, showing an effective IC₅₀ value of 265.75 μ g/mL, while EPSP₂ inhibited α -glucosidase with an IC₅₀ value of 441.87 μ g/mL. In terms of in vitro anti-inflammatory activity, EPSP₁ significantly stabilized HRBCs membrane, with an EC₅₀ value of 8.75 μ g/mL, followed by EPSW₁ (155.17 μ g/mL). Furthermore, the study assessed in vivo anti-inflammatory activity through a carrageenan-induced paw edema model in mice. Simultaneously, the in vivo antidiabetic activity was investigated in Balb/C mice by inducing diabetes via a 1% alloxan injection. Three concentrations (400 mg/kg, 200 mg/kg, 100 mg/kg) were tested for each activity. At 400 mg/kg, EPSP₁ showed the highest anti-inflammatory impact (91.37%) while EPSW₂ at the same concentration displayed significant 74.85% reduction in blood glucose levels in alloxan-induced diabetic mice. In conclusion, the study emphasizes the potent bioactivity of EPS from wheat and Parthenium, showcasing therapeutic benefits, including anti-diabetic and anti-inflammatory properties.

Keywords: Endophyte, Exopolysaccharides, Anti-diabetic, Anti-Inflammatory, Wheat, Parthenium.

Alleviation of Cold Stress in *Oryza sativa* L. by Endophytic Fungi

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ABSTRACT

The purpose of current study is to observe the impacts of cold stress on growth, metabolites and phytohormones of *Oryza sativa* L. and to evaluate the ability of endophytic fungi towards cold tolerance. MSB, MR and DS2 were three endophytic fungi among the six isolated strains which showed considerable potential to mitigate cold stress. The strains considerably increase the amount of total chlorophyll, phytohormones i.e., IAA, GA3 AND ABA, flavonoids, proteins and lipids in plants effected by cold stress. The endophytic fungus (DS2, MR, MSB) inoculation (1gm per 100gm soil) on rice seedlings used. The responses of *Oryza sativa* L. towards the cold stress were described through the analysis of total chlorophyll, xanthophyll, carotenoids, protein, flavonoids, proline, phenolics, sugar, and IAA contents. Under cold stress, growth of rice seedlings was greatly compromised. Cold stress greatly reduced root length (7.6cm) which were markedly increased by endophytes (11.26cm). While shoot length under stress showed 16.23cm which were increased upto 14.86cm. Fresh weight under stress was 0.30gm effectively increased upto 0.42g upon exposure to endophytes. In fugal inoculated ice cold stress was tolerated and growth parameters were significantly improved in comparison to nonendophyte, cold stressed seedlings. Plants treated with cold stress without any endophytic inoculation showed a reduction in growth whereas endophytic fungal inoculation not only restores plant growth and development but also enhances its symbiotic association and showed colonization with host plant roots under cold stress conditions. The isolated endophytic fungal strains were capable of producing secondary metabolites like IAA, flavonoids, phenols and proteins, enables them excellent plant stimulants, so they could be used as bio-fertilizers and bio-remediating agents in cold stress areas. We concluded that the endophytic fungi plays an immense role in increasing the agronomic and biochemical parameters of rice seedlings when exposed to cold stress.

Keywords: *Oryza sativa*, Endophytic, Fungi, phytohormones.

Green Synthesis of Nanoparticles from *Fagonia Cretica* and Evaluating its Anti-Diabetic Activity through *In-Vitro* and *In-Vivo* Analysis

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ABSTRACT

Therapeutic moieties derived from medicinal plants as well as plants-based ecofriendly processes for producing nanoparticles have shown great promise in the management of type 2 diabetes mellitus (T2DM). The current study was aimed to assess the anti-diabetic potentials of *Fagonia cretica* mediated biogenic selenium nanoparticles (FcSeNPs) using *in-vitro* and *in-vivo* approaches. The bio-synthesized FcSeNPs were characterized using various techniques including UV-VIS spectrophotometry and FTIR analysis. The *in-vitro* efficacy of FcSeNPs were assessed against α -glucosidase, α -amylase enzymes as well as the anti-radical studies were performed using DPPH and ABTS free radicals scavenging assays. For *in-vivo* studies, 20 Male Balb/C albino-mice were randomly divided into 4 groups (n = 5) including normal group, disease group (Diabetic group with no treatment), control group and treatment group (Diabetic group treated with FcSeNPs). Further, biochemistry markers including pancreas, liver, kidney and lipid profile were assessed for all treatment groups. The FcSeNPs exhibited a dose-dependent inhibition against α -amylase and α -glucosidase at 62-1000 $\mu\text{g mL}^{-1}$ concentration with IC_{50} values of 92 and 100 $\mu\text{g mL}^{-1}$ respectively. In antioxidant experiments, the FcSeNPs demonstrated significant radicals scavenging effect against DPPH and ABTS radicals. In STZ-induced diabetic mice, a considerable decline in blood glucose level was observed after treatment with FcSeNPs. Anti-hyperglycemic effect of FcSeNPs treated animals were high as compared to standard drug. Biochemical investigations revealed that all biochemical parameters for pancreas, liver function, renal function panel and lipid profile were significantly lowered in FcSeNPs treated animals. Our findings indicate a preliminary multi-target efficacy for FcSeNPs against type-2 diabetes and thus warrant further detailed studies.

Keywords: *F. Cretica*, Diabetes, Oxidative stress, Biogenic, Nanoparticles

Insights into the Morphological Analysis, Phytochemical Profile and Antimicrobial Activities of the Multi-Walled Carbon Nanotubes-Induced *In-Vitro* Sugarcane Culture

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ABSTRACT

The present research work reports the impact of multi-walled carbon nanotubes (MWCNTs) on sugarcane morphological parameters, the production of secondary metabolite (SMs), and the antimicrobial activity of sugarcane callus culture *in vitro*. The induced callifrom leaf sheets were subjected to multiplication and proliferation with the application of various concentrations of MWCNTs (1-5mg/l). The highest multiplication was recorded on media containing 5mg/l of CNTs with fresh weight 2.8567g. Different concentrations (5, 10, 15 and 20 mg/l) of MWCNTs were applied to regenerate the callus. The highest number of shoots and roots were developed on 15mg/l concentration while highest shoot length and root length (in cm) were observed on 20 mg/l CNTs. Similarly, the sub-cultured callus was subjected to phytochemical analysis and the effect of MWCNTs on amount of biochemical contents like i.e total phenolic contents, flavonoids, saponins and terpenoids was determined. Highest content was produced in culture containing highest concentration of CNTs, in all cases. Moreover, the antioxidant activities of the MWCNTs induced *in vitro* sugarcane were also evaluated by the 1, 1 diphenyl 2, picryl hydrazyl (DPPH) methods. The antimicrobial activity was evaluated against two bacterial pathogens, *Clavibactormichiganensis* (CMM) and *Ralstonia solanacearum* (RS) with inhibition zone of 13 mm in case of CMM while 17 mm was calculated against RS Spp. Our results reveal that using MWCNTs in specific concentrations could act as a elicitors of growth and the *in vitro* biosynthesis of useful SMs with antimicrobial activity against broad spectrum microbial pathogens.

Keywords: *Saccharum officinarum*, Multi-walled carbon nanotubes, morphological characteristics, secondary metabolite, antimicrobial activity.

Ligand and Structure Based *In-Silico* Studies to Identify VEGFR-2 Inhibitors as Potential Anticancer Agents

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ABSTARCT

Cancer is characterized as irregular cell growth and division with the potential to infiltrate or propagate to other areas of the body. The role of vascular endothelial growth factor receptor-2 (VEGFR-2) is really crucial in different types of cancer. VEGFR-2 is an angiogenesis regulatory component, deeply implicated in a number of diseases and is also necessary for solid tumor formation. VEGFR-2 autophosphorylation occurs after activation, resulting in endothelial cell proliferation, tumor development, and metastasis. Overexpression of VEGFR-2 is present in a variety of cancers, like cervical cancer, non-small cell lung cancer, colon cancer, and kidney cancer. The recent study attempts to understand the binding mode of known VEGFR-2 tyrosine kinase inhibitors and to design novel and efficient inhibitors of VEGFR-2. A database of 100 compounds collected from thirteen articles was created for this study. The co-crystallized structure of VEGFR-2 (2XIR) was then used to build a Ligand-based Pharmacophore model using the MOE (molecular operating environment) program. The model consists of eight features, two features were designated as essential and the model was verified using the Guner Henry method. The ZINC database was used to screen pharmacophore models, and 340 hits were obtained, which were subsequently employed in molecular docking studies. The binding interactions of the top 200 ranking compounds were detected after docking based on a high docking score. As a result of the good docking score, 10 compounds with key interactions were chosen as novel and potent VEGFR-2 inhibitors for cancer treatment.

Keywords: Cancer, VEGFR-2, Pharmacophore, Virtual screening, Molecular docking

Unveiling the Pharological Applications of Biosynthesized Silver and Nickle Oxide Nanopaticles Using *Lepidium sativum* Mucilage Polysaccharides

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ABSTRACT

Nanotechnology, a rapidly evolving field, focuses on the synthesis, manipulation, and application of nanoscale materials. In recent years, bio-assisted synthesis has gained considerable attention as a means to overcome the limitations associated with conventional physical and chemical methods. This study aimed to synthesize silver (Ag) and nickle oxide (NiO) nanoparticles (NPs) using the water-soluble polysaccharides extracted from *Lepidium sativum* (cress) seed mucilage. Mucilage was extracted by soaking cress seed while polysaccharides were precipitated by treating mucilage with 75% ethanol for overnight. The biosynthesized AgNPs and NiONPs were confirmed through spectroscopic techniques. The UV visible spectrum of the AgNPs gives a sharp absorption peak at 448nm while that of NiONPs was recorded at 343nm. The results revealed that AgNPS was able to inhibit the growth of five human pathogenic *Staphylococcus aureus* (4.5mm ± 0.67mm), *Escherichia coli* (4.12mm ± 1.09mm), *Klebsiella pneumonia* (3.67mm ± 0.19mm), *Salmonella typhi* (5.16mm ± 1.23mm) and *Shigella* (2.9mm ± 0.85mm). AgNPs and NiONPs also exhibited significant antioxidant activities by scavenging the DPPH free radical having with the EC₅₀ value 67.53 and 109.68µg/mL, respectively. These results suggest that water-soluble polysaccharides from cress seed mucilage has the ability to synthesize AgNPs and NiONPs with strong antimicrobial and antioxidant potential and could be exploited for further biomedical applications. This study concludes that utilizing mucilage polysaccharides.

Keywords: Nanoparticles, polysaccharides, *Lepidium sativum*, antioxidant.

Evaluation of the Genotoxic and Cytotoxic Effects of the Selected Medicinal Plants (*Artemisia vulgaris*, *Bougainvillea glabra* and *Ocimum basilicum*)

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ABSTRACT

Medicinal plants are great source of bioactive compounds and chemical structures that have potential beneficial effects. *Artemisia vulgaris*, *Bougainvillea glabra* and *Ocimum basilicum* are important medicinal plants of Pakistan. The role of these medicinal plants has been determined in many therapeutic purposes and have many advantages in pharmaceutical industries including prohibition of cardiovascular diseases, diabetic mellitus, menstrual pain, gastric problems, neuro-degeneration defects, cardiovascular diseases and cancer disease. However, it is also matter of fact that some medicinal plants showed toxic and carcinogenic effects as well. Medicinal plants exert their toxic effects via toxic chemicals known as toxins. Toxins are hazardous chemical substances that cause many infections and illness. When these toxins are exhaust into the organism by inhalation through mouth or nose. The current study was therefore designed to evaluate the cytotoxic and genotoxic effect of the selected medicinal plants. These activities were evaluated using modern day technologies and assays such as cytotoxicity assay, root length inhibition assay and DNA damaging assay. These extracts were toxic to human blood erythrocytes at high concentrations (250µg/mL, 500µg/mL and 1000µg/mL) tested while less toxic at lower concentrations 25µg/mL, 50µg/mL and 100µg/mL and non-toxic at lowest concentrations (1µg/mL, 5µg/mL and 10µg/mL). In root length inhibition assay roots growth was inhibited by all the extracts except *A. vulgaris*. DNA damaging assay showed that the extracts of these compounds have no effect on DNA Damage at lower concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) and showed damaging effects on higher concentrations (250 µg/mL, 500 µg/mL and 1000 µg/mL).

Keywords: *Artemisia vulgaris*, *Bougainvillea glabra*, *Ocimum basilicum*, genotoxic, cytotoxic/bioactive compounds, cancer.

AI-Powered Genetic Profiling for Mastitis Resistance in Cattle

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ABSTRACT

Animals, including cattle, play a crucial role in advancing medical research. Mastitis, a significant issue in the dairy industry, is linked to mammary gland inflammation and has economic and health implications. Cattle's susceptibility to mastitis, similar to human mastitis, offers a unique research opportunity. Identifying genes related to milk production is essential for livestock breeding but traditional methods are time-consuming and expensive. A dedicated gene database and machine learning simplify gene discovery, speeding up research and conserving resources. Developing an artificial intelligence (AI) based approach for gene discovery could revolutionize genomics and genetic research, benefiting both cattle breeding and human disease prediction and treatment. The knowledge acquired may have broader applications beyond mastitis, with the potential to contribute to the prediction and treatment of a range of human diseases. The study aims to develop an AI-driven system that identifies and prioritizes mastitis-related cattle genes through genetic data analysis, literature review, and machine learning techniques. Additionally, it seeks to establish a comprehensive candidate gene database containing gene information, functions, associations, pathways, and links to bioinformatics resources. Cattle data on mastitis susceptibility and resistance will be collected via a systematic review of PubMed and Google Scholar. After a three-phase screening and quality assessment, data validation and verification will ensure accuracy. A Venn diagram will identify common candidate genes, followed by gene and pathway analysis using AI and machine learning. Candidate gene identification and validation will be rigorous. The analysis will encompass statistical and AI-based methods, with the development of a dedicated gene database integrated with bioinformatics resources for improved access and usability.

Keywords: artificial intelligence (AI), candidate gene database, cattle genetic improvement, disease susceptibility, gene discovery, mastitis

Antimicrobial Activity of Silver Nanoparticles Synthesized from Aloe Vera Extract

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ABSTRACT

The prefix “nano” is a Greek prefix that means dwarf and represents 1000 millionth of a meter (10⁻⁹ m). Nanoscience is the study of structures and substances on a nanometer scale ranging from 1 to 100 nm. In current materials science, nanotechnology is the most active area of research. Material science has advanced significantly as a result of nanotechnology, which enables researchers to work with materials at the nanoscale to produce novel goods. It has been suggested that the biosynthesis of nanoparticles using plant extract is an economical, and environmentally benign process. Green nanomaterial synthesis is the most contemporary way of synthesis, even though there are various chemical and physical approaches. We used the Aloe vera plant to synthesize silver nanoparticles (AgNPs) and tested their antibacterial characteristics. UV-Vis spectroscopy, Scanning Electron Microscope (SEM), and X-ray diffraction were used to analyze the produced AgNPs. The XRD peaks, which were indexed, represented the face-centered cubic configuration of AgNp's. The exhibited peak indicates nanocrystalline-structured silver particles. SEM confirmed that the average particle size of synthesized NPs was between 30 nm and 35 nm. The antibacterial activity of our synthesized nanoparticles was tested against bacteria (*Escherichia. E. coli*, *Pseudomonas luteola*, *Bacillus Subtillis*) as a function of nanoparticle concentration and the test was done by Disc diffusion method and antimicrobial activity was found to be quite robust. When the outcomes were compared to the effect of antibiotics like Ciprofloxacin 5, Vancomycin 30, and Ampicillin 10, these antibiotics were shown to be less effective than nanoparticles.

Keywords: Antibacterial Activity, Aloe Vera, Biosynthesis, Nanotechnology, Silver Nanoparticle (AgNp's)

Effect of Selenium Supplement on Immune Responses and the Morphometry of Immune Organs in Broiler under Dexamethasone Induce Stress

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ABSTRACT

The current study evaluated the effect of dietary selenium (Se) on morphology of immune organs and immune responses in broiler chickens reared under dexamethasone (DE) induces stress. Day-old 100 chicks were divided into five groups with four replicates (n=5) in each group. Group A was kept as negative control group provided only basal diet (BD). Group B is considered as a positive control group fed with BD+15mg DE /kg. The group C was fed by Se-0.2mg+15mg DE /kg. Similarly, group D fed by Se- 0.3mg+15mg DE. Group E fed Se-0.4 Se+15mg DE /kg feed. On day 35, two broiler chickens were slaughtered from each replicate. The lymphoid organs samples were collected for tissue processing. The samples of bursa of Fabricius, cecal tonsils, and intestine were processed in paraffin. The sections (5 µm thick) of each sample were stained with H&E stain. In bursa of Fabricius lymphatic follicle length, width and area and in the cecal tonsil, number, length, width, and area of lymphatic nodules were recorded. To assess the humoral immunity the sheep RBC and NDV were used to test antibody responses and for the cellular immune response the phytohemagglutinin (PHA) skin test was performed. The results indicated that supplementation of Se increased ($P \leq 0.05$) the lymphatic follicle number and lymphatic follicle width and area in bursa of Fabricius. The supplementation of Se also significantly increased ($P \leq 0.05$) the lymphatic nodules number and lymphatic nodules width in cecal tonsils. Antibody response against the sheep RBS was significant ($P \leq 0.05$) in broilers. The acidic, mixed and total goblet cells remained significant ($P \leq 0.05$) higher in ileum. Intra epithelial lymphocyte IEL ($p \leq 0.05$) were increased in the ileum, Thus, we concluded that Se improved the morphology of immune organs, immune responses in broiler chickens.

Keywords: BursaCecal tonsil, Dexamethasone, Immunity, Trace element.

Identifying the Role of lncRNA Associated With Glioblastoma by Integrating GWAS and lncRNA

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ABSTRACT

Glioblastoma (GBM) is the most aggressive and highly invasive brain tumor characterized based on its cell origin. It is reported that more than 60% of all the brain tumors are grown-up in GBM. Genome-wide association studies identified 41 dangerous SNPs associated with GBM and these SNPs are mostly lies in non-coding regions. We conducted a multi approach analysis to identify the association of lncRNA SNPs with GBM by integrating them with GWAS for GBM. For the GBM-GWAS associated SNPs, 2000 lncRNA data were utilized to retrieve the proxy SNPs. RegulomeDB was used to functionally rank these SNPs. Overall, 121 SNPs with p-value < 0.05 were functionally prioritized through RegulomeDB. These SNPS were associated with six genes which alter its gene expression. The protein-protein interaction analysis was carried out via STRING. The six predicted genes from these SNPs are PDK4, GARS1, DYP19L3, FISP2, SNHG26 and RNF14P3. Gene Ontology (GO) analysis was carried out via ShinyGO, which shows that these predicted genes were highly enriched in GBM disorder. Moreover, these genes were involved in many biological, molecular and cellular functions. This research demonstrates an indepth view into the proxy SNPs and GBM associated SNPs. We demonstrated that these proxy SNPs were implicated in the GBM-associated gene regulation as well as lncRNA and lncRNAgene interactions. These findings may aid researchers in understanding the process underlying GBM GWAS-associated SNPs.

Keywords: Glioblastoma (GBM), SNPs, lncRNA, GWAS, RegulomeDB, STRING, Gene Ontology (GO), ShinyGO, PDK4, GARS1, DYP19L3, FISP2, SNHG26, RNF14P3

Biosynthesis of Silver and Nickle Oxide Nanoparticles Using Polysaccharides from Chia Seed Mucilage and Evaluating Their Biological Applications

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ABSTRACT

Green synthesis of nanoparticles has gained attention because of their diverse biological applications. The current report tge ecofriendly, and cost effective synthesis of silver (Ag) and nickle oxide (NiO) nanoparticles (NPs) using water-soluble polysaccharides extracted from chia (*Salvia hispanica*) seed mucilage. Chia seeds were soaked in autoclaved distilled water and the polysaccharides were extracted through 75% ethanol precipitation. The biosynthesized AgNPs and NiONps were confirmed through spectroscopic techniques. The UV visible spectrum of the AgNPs gives a sharp absorption peak at 473nm while that of NiONPs was recorded at 343nm. The antibacterial potential of the nanoparticles was evaluated using agar well diffusion method against five different human pathogenic strain. Results revealed that AgNPs were able to inhibit the growth of five human pathogenic *Staphylococcus aureus* (4.5mm ± 0.67mm), *Escherichia coli* (4.12mm ± 1.09mm), *Klebsiella pneumonia* (3.67mm ± 0.19mm), *Salmonella typhi* (5.16mm ± 1.23mm) and *Shigella* (2.9mm ± 0.85mm). The antioxidant potential was evaluated using DPPH radical scavenging assay. It was noted that AgNPs and NiONPs exhibited prominet antioxidant activities by scavenging the DPPH free radical having with the EC₅₀ value 88.53 and 90.23µg/mL, respectively. Furthermore, the invitro antidiabetic activity was evaluated using alpha amylase and alpha glucosidase inhibition assay. It was found that AgNPs and NiONPs showed promising alpha amylase inhibitory potential having EC₅₀ of 76.50µg/mL and 287µg/mL. These results suggest that water-soluble polysaccharides from chia seed mucilage has the potential to synthesized AgNPs and NiONPs with strong antimicrobial and antioxidant potential and could be exploited for further biomedical applications. This study concludes that utilizing mucilage polysaccharides could offers a sustainable source for green synthesis of bioactive nanomaterials.

Keywords: nanoparticles, polysaccharides, Chia, antimicrobial, antioxidant.

Exploring the Regulatory Network of *Arabidopsis thaliana* Phosphate Transporter 2 (*AtPT2*) Gene and its Orthologues in Plants, Using Bioinformatics Approach

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ABSTARCT

Phosphorus plays a crucial role, serving as one of the fundamental inorganic supplements required by living cells and possessing the widest range of functions. The mobilization of phosphate in plants is a complex process that necessitates various transporters for the translocation and absorption of this vital supplement. This study focuses on the bioinformatics characterization of phosphate transporters, specifically *AtPT2* (*AT2G38940*), and their orthologs in different plants. The objective is to elucidate the molecular mechanism governing phosphate transporter function. The current research aims to unveil the gene-regulatory network and molecular basis controlling the expression of *ATPT2* by analyzing the evolutionarily Conserved NonCoding Sequences (CNSs) in the -1000 bp promoter region. Upstream promoter sequences, counted from the translation initiation codon (ATG), were examined. Additionally, the study tested the potassium phosphate stress uptake response of *Cucumis sativus* (*Cucsa.255370.1*) and *Phaseolus vulgaris* (*Phvul.007G193200.1*). Comparative genome-wide bioinformatics analysis, utilizing various tools for different functions, identified evolutionarily preserved regulatory sequences, revealing three highly conserved upstream noncoding sequences (CNSs). Consensus sequences from the conserved sequence logo indicated the positions of consensus sequences: (1) -29bp to -39bp, (2) -55bp to -75bp, and (3) 400bp to -410bp from the ATG in the promoter region of *Arabidopsis thaliana*. The putative cis-regulatory elements identified in the promoter region of the *ATPT2* gene are expected to facilitate the physical binding of upstream regulatory proteins, which are yet to be determined. These novel putative cis-regulatory elements may play a role in controlling the phosphate transporter. Our study suggests that potassium phosphate positively affects *Cucumis sativus* (*Cucsa.255370.1*) and *Phaseolus vulgaris* (*Phvul.007G193200.1*) up to a certain threshold level, beyond which the potassium phosphate concentration is 20 ug/ml.

Keywords: *Arabidopsis thaliana*, *Cucumis sativus*, *Phaseolus vulgaris*, Bioinformatics,

Isolation and Pathological Investigation of *Mycoplasma bovis* in Yaks in District Chitral

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ABSTRACT

Mycoplasmas are minimal self-replicating organisms, but successful and sophisticated pathogens. *Mycoplasma bovis* (*M. bovis*) is one of the causative agent of contagious pleuropneumonia syndrome (CPPS) in adult bovine (cattle, buffaloes, yaks) and calf pneumonia (CP) in the young calves. CPPS results in the enormous economic impact on the cattle industry in terms of production loss i.e. milk and meat. Treatment cost and welfare of the animals are also counted as the important outcomes of this disease. As a member of bovidae family, Yak (*Bos grunniens*) is also susceptible to mycoplasmosis. Yaks are indispensable for the high mountainous pastoralists but very limited studies have been conducted about *M. bovis* in yaks. In our study 200 samples including nasal swabs, lung tissues and milk from *M. bovis* suspected yaks from Yarkhoon (Broghil) and Laspur (Shandur) valley of Chitral district were collected for isolation of the mycoplasma *bovis* and investigate the pathological changes associated with the disease. For the identification and isolation of the organism these samples were cultured in the Pleuropneumonia like organism (PPLO) medium and incubated for 2-5 days at 37°C in 5% CO₂. Purified fried egg colonies of *M. bovis* were isolated from the agar plate on day 5th post incubation. Eleven (11) strains of *M. bovis* were confirmed by PCR using *uvrC* (DNA repair) gene. Fifty-five percent (55%) of the samples collected from Broghil area were culture positive while it was 42% for the samples collected from Laspur. Similarly, PCR positive ratio was also high (14.03%) for the samples of Broghil in comparison with Laspur valley i.e. 6.52%. Lungs from infected yaks showing gross lesions were processed and the histopathological findings revealed mixed patterns of pneumonic lesions. Suppurative bronchopneumonia was seen in 40% of the lung tissue followed by fibrinous bronchopneumonia and caseonecrotic bronchopneumonia 33% and 26% respectively. The results of the present study highlight the importance of *M. bovis* in Yak population.

Keywords: *Mycoplasma bovis*, Isolation, Bovine Respiratory Disease, Polymerase Chain Reaction, Histopathology, Yak

In-Silico Analysis of nsSNPs in *Il2* and *Il3* Genes: Identification of Determinantal Variants and Potential Therapeutic Targets

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ABSTRACT

Rheumatoid Arthritis (RA) is a complex autoimmune disorder with a genetic basis. Interleukin 2 (IL2) and Interleukin 3 (IL3) genes are among the potential candidates due to their associations with RA, Systemic Lupus Erythematosus (SLE), and Diabetes. The study aimed to investigate the impact of nonsynonymous SNPs (nsSNPs) on IL2 and IL3 proteins and evaluate their functional and structural effects. We utilized various computational methods including PROVEAN, SIFT, SNP&GO, PhD-SNP, and PolyPhen2, to achieve this. We further validated our results using I-Mutant, MutPred1.2, and ConSurf servers, which enabled us to examine the stability, functional, and structural effects of nsSNPs obtained from the dbSNP database. Using I-TASSER, we generated 3D models of wild-type and mutant IL2 and IL3 proteins and performed molecular docking analysis. We also compared the three-dimensional structures of the mutant and wild-type proteins using SWISSPDB and performed phylogenetic analysis. We identified six deleterious nsSNPs in IL2 and 10 IL3 proteins, predicted to decrease protein stability and impair function. Results showed that the interaction between Iloprost R-Isomer and normal IL2 protein involves Leu73 and Thr151, while in the case of the mutant, it involves Leu71 and Thr151. Similarly, for IL3 gene, the interaction between 1-but-2-ynoxy-4 and normal IL3 protein involves Pro27, Thr30, Trp32, Cys35, Arg74, Leu106, and Gln141, whereas in the case of the mutant, it involves Pro27, Thr30, Trp32, Cys35, Leu106, and Gln141. The molecular docking analysis of the IL2 gene revealed that Gene-gene interaction analysis revealed the significance of IL2 and IL3 in various pathways. Our findings suggest that consideration of these damaging nsSNPs can be valuable in precision medicine for diseases related to IL2 and IL3.

Keywords: Rheumatoid Arthritis (RA), Interleukin 2 (IL2), Interleukin 3 (IL3), Nonsynonymous SNPs (nsSNPs), Protein Stability, Molecular Docking

New Findings on the Human *Trypanosoma evansi* Infection

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ABSTRACT

Trypanosoma evansi, the causative agent of 'surra' kills thousands of animals every year and causes significant animal morbidity and loss of productivity. This flagellated hemoprotozoan parasite originated from *Trypanosoma brucei* through deletion of the maxicircle kinetoplast DNA which conferred the capacity for mechanical transmission by flies and allowed *T. evansi* to expand beyond the tsetse belt. Presently, it is the most widely distributed pathogenic trypanosome in Africa, Asia, and Latin America, but its potential for geographical extension is not limited, as shown by recent sporadic cases in Spain and France. Human infections with *T. evansi* have been reported from India, Vietnam, Sri Lanka, Egypt and Indonesia. For a decade it was hypothesized that human susceptibility to *T. evansi* could be linked to insufficient or missing levels of human trypanocide apolipoprotein L1 (APOL1), a trypanocidal component of normal human serum. However, a report of infection in a Vietnamese patient in 2016 with no previous immunological risk, 2 wild-type APOL1 alleles and a normal serum APOL1 concentration suggested that *T. evansi* is a true zoonosis with a risk of infection for the general population. In 2022, a groundbreaking epidemiological study from India revolutionized our understanding of this zoonotic parasite. In that study, 5.2% of asymptomatic individuals tested positive in the CATT serologic test and 2.9% were PCR positive, indicating active but silent *T. evansi* infection in an endemic area. In this presentation, new findings on *T. evansi* infection in humans will be discussed.

Keywords: atypical human trypanosomosis, diagnosis, human African trypanosomosis, One Health, *Trypanosoma evansi*, surra, zoonosis

HEALTH SECURITY AND BIOSECURITY

HB-1

Patient Safety Culture among Health Care Providers at Kabul University of Medical Sciences Abu Ali Ibn Sina Educational Hospitals

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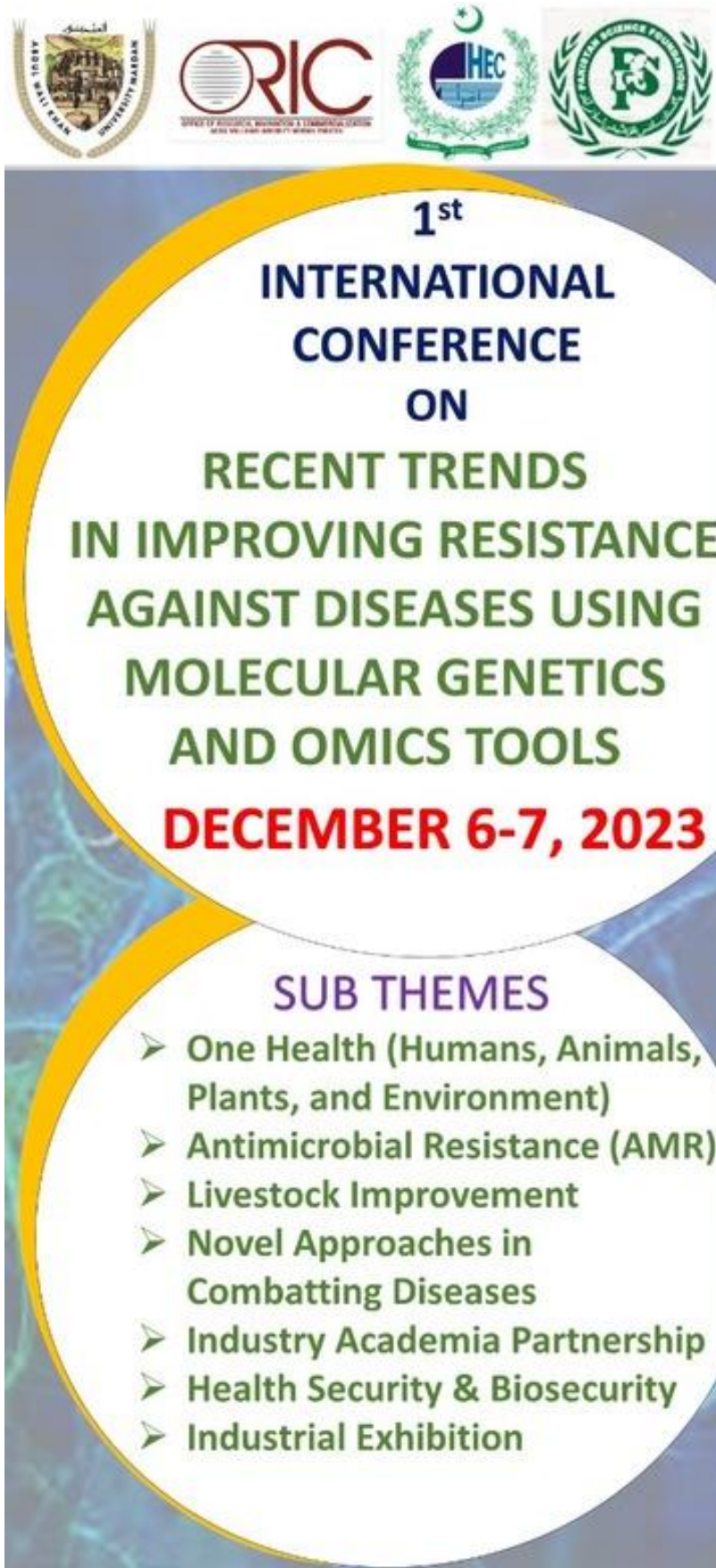
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Abstract

In the field of healthcare, the well-being of patients is often compromised by instances of unsafe care or medical errors, leading to additional costs for both individuals and healthcare systems. Enhancing and fostering a culture of patient safety within the healthcare industry plays a crucial role in enhancing the quality of care provided to patients. To assess the current state of patient safety culture among health care providers at Kabul University of Medical Sciences Abu Ali Ibn Sina educational hospitals. A descriptive cross-sectional study was conducted from June 2023 to September 2023 at the Kabul University of Medical Sciences Abu Ali Ibn Sina educational hospitals. The data were collected using the Persian version of the standard Patient Safety Culture Survey questionnaire, which consisted of 12 dimensions. Among 282 health care workers, 270 completed the study, with a response rate of 95.74%. From the respondents, 205 (75.9%) were males, and the mean age was 33.3 (\pm 9.2) years. According to 12 aspects of patient safety culture, two indices of teamwork within hospital units and organizational learning (continuous improvement) were among the high-level positive responses, with average positive response rates of 78% and 64%, respectively. On the other hand, non-punitive responses to errors and hospital handoffs and transitions were at the lowest levels, with average positive response rates of 35.8% and 36.7%, respectively. The total mean patient safety culture in understudy hospitals was 51.78; in this study, 53.3% never reported at least one event in the last 12 months. In the hospitals under investigation, patient safety was at an average level. It is suggested that hospitals emphasize patient safety issues more. The areas that had the lowest average ratings should receive special attention in order to strengthen them.

Keywords: patient safety, patient safety culture, Educational hospital, *Kabul University of Medical Science*.



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