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Tangible And Intangible Returns – Finding A Right Narrative For Research In Nepal

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Abstract:

As the number of patents filed from Nepal is extremely low, immediate commercial return on investment in research might not be a convincing narrative. However, the number of ranked scientific publications coming from Nepal, both in absolute number and as a ratio to national GDP is quite impressive. A narrative based on the research output, particularly its foundational importance and multifaceted impact on education and socioeconomic climate might be a more realistic and convincing narrative. Link of research priority, funding and quality assurance to research output, and its importance is discussed.

Keywords: Output, Research funding, Return on research

Biobanking In Finland

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Abstract:

Human biological samples and health data for medical research have been collected throughout the history of medicine. Methods for collecting and storing samples, as well as for consenting sample donors, have varied greatly over the years depending on the current legislation and other policies. In 2013, Finland enacted a Biobank Act. According to it, a biobank is a collection of samples and associated data gathered with donor's consent for future research purposes.

Finland is regarded as a successful environment for medical research and biobanking. This is mainly due to four things: 1) population based, high coverage public healthcare system; 2) unique national identification number for each citizen; 3) over 175 registries that can be utilized in scientific research; and 4) genetically isolated population. At the moment, ten biobanks operate in Finland. Most of them are hospital-integrated, collaborative efforts between the local university and university hospital. By the end of 2017, more than 125000 Finns had given their consent for biobanking. The main sample type collected is 10 ml EDTA blood that is processed into plasma and DNA. Additionally, millions of diagnostic formalin-fixed paraffin embedded tissue samples collected between 1980 and 2013 has been transferred into biobanks to form a remarkable source for tissue-based research.

Samples and data in Finnish biobanks are open to all, whether the applicant is Finnish or foreigner, an academic or industrial researcher. Sample access is based on a written application, evaluated by an ethics committee. After a favorable decision, a material transfer agreement is written. To date, Finnish biobanks have delivered material for more than 300 studies.

The advantages of biobanking are clear. Biobanks accelerate research by providing a readily accessible resource of well-annotated, high quality samples, which reduces the amount of time researchers; need to spend recruiting volunteers to build a sufficient cohort for their studies. In addition, biobanked materials are collected in a standardized and coded manner ensuring patient confidentiality as well as meeting legal and ethical requirements. I have been closely involved in establishing three different Finnish biobanks between 2011 and 2017. In my presentation, in addition to things mentioned above, I will describe how to get started and what are the 10 most important things to consider when establishing a biobank. I will also present more in detail, how Finnish biobanks are operating and what their future goals are.

Keywords: Biobanking, Hospital-integrated, Patient samples, Finnish policies

Translational Research And Modern Biobanking: Examples From Helsinki Biobank And Helsinki Urological Biobank

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Abstract:

Finland, through biobanking offers some truly unique resources to conduct high-impact medical research. Easy, ethical, and economical access to patient samples for translational research has been a challenge world-wide. While current biobanking efforts are reducing the barrier for researchers to access patient samples, another challenge is ethical access to clinical data associated with those samples. Lastly, while many in the research and medical communities are aware that biobanks exist, intuitive examples and applications of their use are not well understood amongst the community. Since 2012, I've worked in the research group of Dr.TuomasMirtti and DrAntti Rannikko, and have been focused on clinical-translational applications of biobanked patient samples. We strive to conduct high level translational and clinical research to improve patient outcomes of urological malignancies, specifically prostate cancer. Our studies use high quality retrospective studies utilizing patient-specific local hospital and national registry real world. Our aim is to improve the quality of hypothesis testing to feed forward into prospective and/or randomized clinical trials.

Since 2017, I joined the Helsinki Biobank, in part time position, as a project manager. My work in the Helsinki Biobank relies on applying data science skills to identify patient cohorts, specifically for retrospective, archival biobanked samples for over 30 potential applicants. Furthermore, I have presented biobank work previously in other international conferences, leading to some of the first external international academic biobank applications to the Helsinki Biobank. Please don't hesitate to contact me if you'd wish to discuss practical, potential use of biobanks in your work.

Keywords: Biobank, Real World Data, Urology, Pathology, Prostate cancer

Cancer Burden And Cancer Control Strategy In Nepal

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Abstract:

Nepal is a resource-limited country in South Asia with a population of 29 million and approximately 30,000 new cancer patients every year. With a diverse culture and ethnicity, and the difficult terrain in Nepal, accessibility to health care makes cancer care difficult. As there is an increasing trend of cancer in Nepal, cancer control programs (CCPs) have also been on the rise over past decades. CCPs should provide evidence-based cancer services, which are safe, standard and cost-effective.

According to hospital based national cancer registry in Nepal, the most common cancer sites are lung, cervix, breast, stomach and larynx for both sexes. Infection-related cancers (HPV, H. pylori and Hepatitis) are more common in our subcontinent. There is strong evidence that vaccination or treatment prevents these cancers. Other than smoking, intake of betel nut, tobacco chewing, excessive consumption of alcohol and increasing trend of eating junk food are common risk factors of cancer in the country. Taxation on tobacco, alcohol and ban on advertisement can lead to reduction in their use. Moreover, we have to enhance the health promotional activities to prevent common NCDs including cancer in Nepal.

Main cancer diagnostic modalities are available in both public and private hospitals of Nepal. Recent imaging technologies including PET-CT are available in the country. Cancer surgeries and chemotherapy have been available in most of the major hospitals that are located in urban areas. Radiotherapy service is available in six centers and more high quality services are needed in public setup. An evidence-based national cancer management protocol should be practiced in the country. Cancer research in Nepal is limited only in the form of retrospective case series and case report publications.

Asymptomatic cancers are rarely diagnosed in Nepal due to absence of mass screening and health insurance. More than 80% of the cancer patients are in advanced stage and all of them need palliative care. Hospice service in a few Nursing home or hospital inpatient settings has been available since 2000. Nepal has been categorized recently as 'generalized palliative care provision' in South Asia by the global classification of palliative care provision.

In conclusion, public awareness, prevention, treatment and research related to cancer should be based on the available evidences. Public and private partnership is needed to better diagnose and treat cancer in Nepal.

Keywords: Cancer burden, Cancer control strategy, Palliative care, Prevention

Approaches On Drug Discovery From Nepalese Plants

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Abstract:

Nepal has a rich tradition of plant-based knowledge on healthcare. A large number of plants, plant extracts, pastes and plant powders are used by tribals and folklore traditions in Nepal for treatment of several types of disease. We validated the plant based traditional medicines and prepared their different doses forms such as tablet, capsule, ointment, cream, gel, microsphere, transdermal patches, etc by taking knowledge of long history of herbal usage for the clinical management of a variety of diseases in indigenous cultures on Nepal. The major pharmacological strategies such as phytochemical and antimicrobial screenings, antioxidant, wound healing, analgesic, antidiabetic, anti-inflammatory properties of plants have been carried out in the discovery of herbal formulation of potential clinical value.

Mango and orange peel showed remarkable results in cancer cells, which could be a very potential anticancer agent against human cervical carcinoma and gastric carcinoma and cervical carcinoma both respectively. *Diploknema butyracea* seed oil and its formulated 5% ointment showed significant effect as analgesic, anti-inflammatory and wound healing agents. *Psidium guajava* leaf extract can be considered for transdermal patch for better release for the management of diabetes. Similarly, extract of *Urtica dioica* showed satisfactory result as antidiabetic drug in Streptozotocin induced diabetic mice. Similarly, ointment prepared by fusion method from *Gaultheria fragrantissima* oil showed analgesic effect, spread ability, stability tests but negative result for irritancy test. Plants screened for high tannin and phenolic contents with significant antioxidant property were formulated into wound healing ointment. 10% w/w of *Bauhinia variagata*, *Rhododendron arboretum*, *Myrica esculenta* ointment found to be more effective in healing wound than 1% w/w Framycetin cream. Furthermore, in another experiment *Camellia sinensis*, *Punica granatum*, *Hordium vulgare* plant extract showed potent anticancer activity. LD50 of *C. sinensis* decreases from 24 hrs to 48 hrs for both MCF-7 and MDA-MB-231 cancer cell lines. In addition, microsphere loaded gel of *Lactuca sativa* is prepared by dispersion and then formulated for 2% gel and 4% gel, which showed potential anti-inflammatory activity, anxiolytic activity, anti-oxidant activity and anti-microbial activity. Conclusively, medicinal plants of Nepal are very important for the aspect of their anticancer, wound healing, analgesic, anti-inflammatory, anxiolytic and antimicrobial properties. These results will be useful in the validation of the clinical application of above mentioned herbs and the development of novel herbal therapeutics from the same.

Key words: Himalayan plants, Ethnobotany, Bioactivity, Formulation, Drug development.

Discovery Of Potent Protein Tyrosine Phosphatase 1B Inhibitor In Vitro From The Medicinal Plants Of Nepal

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Abstract:

The health calamity caused by obesity throughout the world is a burning issue in the sedentary lifestyle. Obesity heightens the risk of associated morbidities, including diabetes, hypertension, cancer, heart disease and so on. Overexpression of Protein tyrosine phosphatase 1B (PTP1B) enzyme is responsible for type 2 diabetes and obesity. Numerous anti-diabetic synthetic drugs are available in the market but not away from severe side effects. So, the research is focused on identification of the potent plants extracts that exhibit inhibitory activity against PTP1B enzyme. Regarding the goal, ten plants extracts were tested to inhibit the activity of PTP1B enzyme. Some of the extracts were showed remarkable inhibition percentage (60-80%) against PTP1B by pNPP assay.

Keywords: Inhibition percentage, Plant extract, pNPP assay, PTP1B, Type 2 Diabetes

Department Of Biotechnology And It's Activities In Kathmandu University

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Abstract:

Department of Biotechnology, first of its kind in Nepal, was established at Kathmandu University in 2003 in view of responding to a sharp shortage of graduate biotechnologists in the national bio-industries and allied areas with a mission to cater to and supply a steady stream of highly competent biotechnology graduates required for the country and contribute to generating new knowledge in the field of biotechnology. It is overviewed that the graduates from the Department of Biotechnology will be equipped with both theoretical knowledge and practical experience to pursue higher studies and enter into job markets in Nepal and overseas. In objective to prepare students to face the challenges ahead in view of the emerging needs for new knowledge and skills generated from innovation and experience, this department will strive to make sure that students that graduate from KU with a biotechnology degree are recognized at the national and international levels and that their knowledge will enhance biotechnology worldwide. The prioritized area of research under this department includes

1. Natural Product research: Research related to medicinal plants and soil microbes
2. Molecular Biotechnology: Research related to Molecular analysis of plant and bacteria such as barcoding of national priorities Herbs, bacterial sequence analysis etc.
3. Bioinformatics: Use of software's for data analysis.
4. Downstream research: Use of Fermentation techniques and isolation of enzymes for different food and other biotech based industries.
5. Immunology research: Development of antibodies and data analysis by immunologic assays.
6. Microbiology research: Identification, characterization and effect analysis of various bacteria and their activities and resistance performances.

Keywords: Kathmandu University, Biotechnology, Research

An Overview Of Scientific Publications In Nepal

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Abstract:

Publication of research outputs in peer reviewed journals usually reflects both the quality and quantity of research activities in a given institution and country. In this work we aimed to understand the dynamics of scientific research productivity in Nepal. We analyzed publication data (>60 years) obtained from Scopus database. Nepal has relatively a short history of substantial scientific research. The number of scientific publication is increasing over the years, with about 1000 publications in the year of 2016. Interestingly, publications from medicine dominate among many research fields. In this presentation, we will discuss the trends of scientific publications, major institutions & research fields, and aspects of international collaboration in Nepal.

Keywords: Scientific research, Publication, Nepal

Diversity And Applications Of Bacterial Natural Products

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Abstract:

Bacterial natural products have been valuable tool for deciphering the logic of biosynthesis and making platforms for developing front-line drugs. Natural products and their derivatives as antibiotics have saved the lives of millions for decades. Screening of new bacterium for discovery of novel lead compounds is essential as delivery of the new drugs for future use demands discovery pipeline to be full. Most of small molecules from bacteria are secondary metabolites which are products of conditional pathways that are turned on in a particular context or situation (during starvation, development, signaling, etc.). Exploiting the bacteria for production of the entities serving mankind has been possible due to its advantages over other organisms. Those advantages are: ease of growing, engineering and upscaling the metabolite production, well-known genetics due to its small genome size and tunable regulatory systems. Moreover, achievements in systems and synthetic biology have made bacteria a wonderful chassis for designing and customizing the genetic circuits for rewiring heterologous biosynthetic pathways of secondary metabolites. The bacteria as a host is providing enormous platform for biosynthesis of important natural products serving as medicine, food, fuel and cosmetics.

Keywords: Natural products, Bacteria, Pathway engineering, Secondary metabolites biosynthesis

Epidemiological studies in Finland

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Abstract:

Finland has a long tradition of epidemiological studies from the 1950s onwards. These have been enabled by a number of characteristics of the country. There has been a systematic registration of the population since the 1600s, such that local parishes recorded all births, deaths, marriages and moves to and from the parish. Since the 1960s this information has been computerised and all Finnish citizens are assigned a unique personal identifying number (PIN) based on date of birth. The PIN is widely used in health care and medical registries, thus enabling comprehensive and unbiased follow-up of participants in epidemiological studies even over decades. Further, the PIN permits linkage of various medical registries (such as the Cancer Registry, the National Prescription registry, the register of all hospital inpatient and outpatient visits) to identify incident cases of disease in follow-up cohorts.

Many cohorts that represent the Finnish population have been established since the 1960s to study determinants of disease. Examples include the Finnish part of the Seven Countries Study, the North Karelia project and its continuation as the FINRISK surveys, the Health2000 study, the ATBC intervention study and the FINGER study. Participation rates have generally been very high, indicating the positive attitude of the population.

Since the 1950s the health of the Finnish population has vastly improved, with life expectancy increasing among Finnish men by about 20 years over a 6-7 decade period since WWII; the epidemiological studies have contributed to this development as results have been taken into practice in public health policy and clinical work. Starting in the 1990s, the study cohorts have also collected DNA, which has been increasingly genotyped and used in multiple ways to assess the contribution of genes in disease incidence and risk factors for health.

Keywords: Epidemiology, Cohorts, Finland, Register, Health monitoring

Opportunities And Challenges Of Conducting Epidemiological Studies In Nepal

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Abstract:

Nepal has made significant strides in health in the past few decades. The rapid growth of academia in health sciences related sectors is one of the drivers of this change. Concurrent to this growth of academia, health research has also been expanding. Epidemiological studies are the foundations for health research as they give comprehensive overviews on the scenario of disease burden as well as their risk factors in the population. Historically, most of the epidemiological inferences have been made from the studies in developed countries. There is an increasing need and opportunity to generate (and utilize) our own data and get insights into the uniqueness of health issues of our population. Lack of funding, difficulties in coalescing various groups of health researchers, and gaps in capacity-building programs are major challenges in advancing epidemiological studies in Nepal. However, these can be addressed by proactively improving coordination and collaboration with various stakeholders in health sector, developing common and shared platforms for capacity building on epidemiological research, and establishing consolidated ties with international. We should be bothered not by the magnitude of the challenges we face but rather by the ignorance of the opportunities we have.

Keywords: Epidemiological Studies, Nepal



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Deciphering The Role Of PQBP1 As A Tumor Suppressor Gene In Prostate Cancer Cell Line DU145.

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Abstract:

Polyglutamine Binding Protein-1(PQBP 1) is encoded by a gene present on X chromosome. It is an important protein in terms of its role in Neuro-degeneration which leads to X-Linked Mental Retardation. It is a compelling hypothesis that proteins which play role in euro-degeneration induce apoptosis. Various evidence has been found to support of this as over expression of this protein in transgenic mice and in vitro samples have induced apoptosis and late motor neurone development. We speculate that this protein might also play a significant role in cancer cell proliferation. Phenotypic studies done on DU145 cells of over expression and knock down shows that PQBP1 inhibits cell proliferation. Though in vitro assays of over expression and knockdown of PQBP1 in prostate cancer cell line DU145, it down regulate protein involved in inducing cell proliferation like Pin1, C-Jun, CMyc, phosphorus-cMyc, NF-KB and P38. Moreover it down regulates antiapoptotic protein Bcl-xL after over expression of PQBP1. Hence it can be concluded that PQBP1 can control cell proliferation and induce apoptosis, which are characteristic of tumor suppressor genes. When we overexposed PQBP1 colony size of cells has been inhibited. PQBP1 could be a novel drug target for the treatment of cancer, could be developed as cancer biomarker for future therapy.

Key words: Cancer, Protein-Protein Interaction, PQBP1.

Rapid detection of Tuberculosis using Magnetic Nanotechnology Particles (MNPs) in Nepal

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Abstract:

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is an extremely dangerous and ubiquitous bacterium. This presentation describes new technology for rapid detection of Mtb with the use of nanotechnology. With the significant morbidity that is associated with tuberculosis its infectivity, it is imperative that quicker detection be available. Therein lies the opportunity for magnetic nanoparticles (MNPs) to detect tuberculosis in patients quickly and at an estimated cost of USD 0.05 per test.

Dhulikhel Hospital partnered with the Michigan State University USA, to collect a total of 390 sputum samples. The aggregation of Mtb to the MNPs aids in the efficiency and accuracy of microscopic identification. Sputum samples were obtained from patients suspected of TB based on a thorough history and physical exam. The use of MNPs in attempting to detect Mtb yielded a sensitivity of 100 % and specificity of 100%. Microscopy with the use of Ziehl-Nielsen staining yielded a sensitivity of 60% and specificity of 42%.

Our study has shown substantial improvement in the sensitivity and specificity of the detection of Tuberculosis. This is an important for the fields of public health, medicine, and biodefense. Nano technology opens the door for a reliable screening method for TB with the possibility for uses in various clinical settings with a wide range of available resources.

Keywords: *Mycobacterium tuberculosis*, Magnetic nanoparticles, Microscopy, Ziehl-Nielsen staining

Onco-Immunological And Tumor-Stromal Assays For Precision Medicine Evolution

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Abstract:

Cancer precision medicine aims to provide optimal treatment solutions for each patient based on individual tumor drug response profiling combined with available molecular and genetic hallmarks, as well as prior patient disease and treatment history. However, current assay platforms at the Institute for Molecular Medicine Finland (FIMM) rarely take into consideration the impact various treatments have on the immune system, nor do they include stromal cells or other factors of the tumor microenvironment potentially influencing treatment efficacy.

To gear the FIMM precision medicine machinery towards a more holistic approach that also incorporates components of the host immune system and/or tumor microenvironment, we have been developing new assays and detection methods amenable for high-throughput screening. Preliminary findings indicate that immune cell functions may be either inhibited or enhanced by oncological drugs, and stromal-tumor co-cultures have revealed effects on drug resistance that were not evident in monotypic cultures. Several technical hurdles remain to be overcome but these promising findings pave the way for the next generation of precision medicine platforms at FIMM.

Keywords: Oncoimmunology, Stromal co-culture, Functional tumor killing assay, Immunotherapy, Immunomodulation, Image cytometry

Tackling Functional Heterogeneity In Non-Small Cell Lung Cancer

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Abstract:

Tumours behave as ecosystems in which gene-environment interactions constitute a dynamic equilibrium that evolves over time. Using immune competent mouse models, we study gene-phenotype relationships across the histopathological diversity of non-small lung cancer (NSCLC) tumours. We find that while genetic drivers define histopathology spectra, precise spectra are influenced by the tumour's cell of origin. Importantly, phenotypic diversity in oncogenic signalling and immune microenvironments align more closely with histotype, rather than driver genotype. This determines histotype-specific adaptive resistance mechanisms, and (combinatorial) drug sensitivity. Finally, using tumour explants, we show that responses to combination treatment with signalling inhibitors correspond with spatially-defined targeted pathway activities. Our work implies the existence of NSCLC histopathology-specific phenotypes, and cautions against an over-reliance on genetic markers in personalised diagnostic settings.

Keywords: Lung cancer, Heterogeneity, Histopathology, Cell of origin, Phenotype

Predictive Modelling Of Drug Treatment Responses For Personalized Oncology

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Abstract:

Comprehensive testing of the sensitivity of patient-derived cancer samples to a wide collection of chemical compounds is increasingly being used to tailor drug treatments for individual cancer patients. However, high-throughput drug testing experiments result in high-dimensional datasets, with inherent measurement noise and technical variability, which hinders detection of differential drug sensitivities or stratification of patients based on their response patterns. This talk describes the computational analysis pipeline developed and used at FIMM, which enables reliable quantification of differential drug sensitivities, mapping of target addiction networks behind the individual response profiles, prediction of targeted drug combinations for relapsed patients, as well as identification of biomarkers predictive of selective drug responses. A specific focus is placed on multivariate machine learning models that enable integration of multi-omics data with biological information in terms of target networks when mining for biomarker panels most predictive of treatment responses. Several examples are given, both in hematological cancers as well as in solid tumors, illustrating how these computational models provide experimentally-validated predictions, identify the omics profiles with the highest predictive signal, as well as guide future clinical trials by combining real world registry data and biobanks for prognostic predictions.

Keywords: Predictive modelling, Machine learning, Big data, Biobanks, Biomarkers and Clinical translation.

From Molecular To Precision Medicine At FIMM: A Health Revolution?

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Abstract:

FIMM began in 2006 as a new kind of international research institute, to enable the integration, analysis and translation of genomic, molecular and health data, in order to improve the precise diagnosis and treatment of disease, and to benefit public health. Since that time, a major global shift in healthcare has begun, to meet the problems of aging populations and exploding healthcare costs. This shift is often summarized as 'P4' medicine - predictive, preventive, personalized, and participatory. We will look at how FIMM anticipated this shift in the paradigm of healthcare, in particular through the Digital Health Revolution (DHR) personalized medicine pilot study. DHR combined the digital footprint of individual participants with their genomic and clinical health data, to develop new approaches to healthcare. A central goal was to return control of health data to the individual, and integrate it across healthcare systems and services. We outline the methods used, the problems encountered, and the results.

Keywords: Personalized medicine, Precision medicine, P4 medicine, Healthcare, Research institutions

Genomics At Deerwalk

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Abstract:

The Genomics Department at Deerwalk Services Pvt. Ltd., Kathmandu falls under the umbrella of Deerwalk Group of Companies which includes Deerwalk Institute of Technology (DWIT), Deerwalk Sifal School, Deerwalk Compware Ltd. and others. The Genomics Department at Deerwalk provides a set of specialized services which includes the analysis and curation of sequence variants and the validation of clinico-genetic contents of the software designed to perform such analysis and curation. The team has been conducting these tasks since 2006 and has delivered high-quality analysis and research reports in a variety of clinical settings. The educational background of the team is primarily based on Life Sciences and Biotechnology. They possess high level of proficiency in sequencing technologies and abide by the strict adherence to privacy standards which has helped in maintaining Deerwalk's commitment to quality service.

Genomics at Deerwalk supports any types of genetic diagnostic or research centers engaged in DNA sequencing, analysis of genetic changes to determine impact on disease, or providing software solutions for genomics data. Other key advantages for organizations utilizing the service include rapid turn-around time and quality deliverables. Typical users are clinical/genetic laboratory directors, clinicians, scientists, genetic counselors or anyone building a personal genetic database. Commercial DNA testing laboratories and organizations engaged in research, personalized gene therapy or pharmacology can utilize and benefit from the services provided. Deerwalk also possesses the in-house resources for building and maintenance of any customized tools, databases and/or software that can be beneficial for the analysis of genetic data and curation of sequence variants.

Keywords: Curation, Genomics, Sequence variants, Software.

Multidimensional Genetic Analysis Of Adrenal Tumours, Towards Individualized Precision Medicine

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Abstract:

Malignant adrenocortical tumours are rare and usually an aggressive malignancy with poor prognosis. Multi-omics studies have identified frequent mutations and aberrations occurring in these tumours. However, spatial and temporal analyses of the tumour warrant better understanding of tumorigenesis and tumour progression. Mutation status, copy number variation analysis utilizing high density SNP array, expression analysis at RNA and protein level were performed on multiregional samples of each individual tumour to analyze the possible heterogeneity occurring in these tumours. Results were then correlated with clinical parameters and survival. Phylogenetic trees inferred from copy number variation (CNV) pattern, determination of ploidy levels and mutation status confirmed spatial and temporal heterogeneity. This phenomenon may explain partial response of these tumours to pharmaceutical intervention and high rate of recurrence.

Keywords: Adrenocortical tumours, SNP, heterogeneity

Identification Of Combinatorial Drug Therapy Against Triple Negative Breast Cancer (TNBC) And Pancreatic Ductal Adeno Carcinoma (PDAC) Using A Functional Profiling Approach.

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Abstract:

Most solid tumors still remain therapeutically challenging due to the lack of targeted therapies as well as the development of resistance to targeted mono-therapies. To date, the linear strategy of oncogene to targeted drug has not been very successful in translating basic discoveries to long lasting clinical therapies. However, the availability of a broad array of targeted oncology drugs gives us an unprecedented opportunity to establish a drug sensitivity-based functional profiling of cancers and identify functional disease stratification and novel therapeutic strategies. I choose to work with triple negative breast cancer (TNBC) and pancreatic ductal adenocarcinoma (PDAC) as both types of cancers lack effective targeted therapies. The main focus of the project is to establish functional profiling of both cancer types and identify stratified vulnerabilities that can be targeted with selective drug combinations for each individual or group of individuals. Furthermore, such stratified disease-specific vulnerabilities are linked to molecular profiling and identification of the predictive biomarkers may be applied in clinical settings in future.

Triple negative breast cancer (TNBC) is a highly aggressive disease which affects approximately 15-20% of breast cancer patients. Targeted therapy remains to be established for TNBC that lacks estrogen, progesterone and HER2 receptors, and therefore fails to respond to hormonal and anti-HER2 treatment. This limits the therapy to traditional chemotherapy, radiation and surgery, which is only beneficial to a fraction of TNBC patients. Transcriptomics-based subtyping of TNBC into six classes highlights the diversity within the TNBC diseases. However, it is unclear how the transcriptomics-based subtypes link to effective therapeutic strategies.

Similarly, pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid tumors and one of the leading causes of cancer death. PDAC has poorest prognosis with a median survival of less than 6 months and a 5-year survival rate of less than 5%. Only one targeted drug (erlotinib; tyrosine kinase inhibitor targeting EGFR) has been approved for PDAC which has showed clinically insignificant impact. PDAC is genetically a heterogeneous disease. Although 95% of pancreatic cancers carry KRAS mutations, effective anti-KRAS treatment remains to be discovered. The RAF/MEK/ERK and PI3K/AKT/mTOR pathways are well-validated canonical effector pathways in KRAS-mutant cancer. Hence, the combination approach rendering these pathways might lead to the effective targeted therapy against PDAC.

Key words: Breast cancer, Pancreatic cancer, High throughput drug screening, Combination therapy

Targeting Cancers using Chemical Systems Medicine

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Abstract:

The concept of targeting cancers strictly based on their genetic alterations has gained massive attention in recent years. However, genetics-driven cancer precision medicine clinical trials attempting to broadly link oncogenic alterations with targeted treatments have yet to show significant clinical impact. In an attempt to address these current shortcomings in genomic cancer precision medicine, we have established a platform where we study primary cancer samples by combining comprehensive functional chemo-sensitivity profiling, deep molecular and genetic profiling and clinical information with the aims to allow us understand the linkages between the molecular profile of a cancer and drug sensitivities and to allow for rapid and efficient identification of precision therapies for current and future patients. In having profiled more than 200 acute myeloid leukemia and other myeloid neoplasm cases, we have learnt that the vast majority of selective drug responses are not directly linked to individual genetic alterations and that selective cancer cell killing effects are rarely seen with single agents. Our profiling has on the other hand allowed us to identify new potential targeted uses for approved and investigational drugs such as the VEGFR inhibitor axitinib, which unexpectedly targets a drug resistant gatekeeper mutation of the BCR-ABL oncoprotein. Furthermore, the compiled information allows us to search for more complex biomarkers that may predict clinical drug responses in an unbiased manner. I will present our approaches, some of our findings as well as some of the challenges that lie ahead.

Keywords: Chemical system medicine, BCR-ABL oncoprotein, Biomarkers

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Micropropagation Of Bambusabalcooa Roxb. Of Nepal

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Abstract:

Bambusabalcooa Roxb. is one of the fastest growing monocarpic grass plants. It has a high ability to sequester atmospheric carbon consequently and mitigate climate change. 98% bud break was observed after one week of inoculation in MS liquid media containing 1 mg/L 6-Benzylaminopurine (BAP) with 2 mg/L Gentamycin. Better shoots multiplication was found in MS medium with 3% sugar, 0.01% inositol with 3 mg/L BAP. Sub-culture of shoots was achieved at proliferation rate of 4.5 ± 3.2 folds. Shoot clusters were rooted in MS liquid media without supplement of root hormone after pulse treatment in MS semisolid media supplemented with 5 mg/L Naphthalene acetic acid within a week showed 85% rooting rate in overall culture. 83% survival rate was achieved during primary hardening in sand and the acclimatization rate in secondary hardening was found 90% in the polybag with the composition of sand: soil: vermi compost: ash in the ratio of 1:1:1:1. The protocol achieved multiplication rate of 4.5 ± 3.2 fold shoot in liquid media. The survival rate 86.66% was found by using vermicompost and ash for acclimatization of Bambusabalcooa Roxb.

Keywords: BAP, Bambusabalcooa, Liquid Media, Root, Shoot

Treatment Of Agricultural Waste And Generation Of Electricity In Microbial Fuel Cell

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Abstract:

Agricultural solid waste is a great problem in waste management sector. There are different techniques which are used for agriculture waste management however if there is production of value added products, that will be of additional benefit. Microbial fuel cells (MFCs) are devices that uses microbial metabolism to degrade organic waste from a wide range organic substrates. Alternatively, they produce electricity from microbial metabolism. So, use of an efficient microbial fuel cell for proper management of organic waste of different composition and then comparing its degradation efficiency will generate electricity.

Chemical oxygen demand (COD), reducing sugar contents, phosphorus contents of agricultural waste were determined. Two chambered microbial fuel cell was constructed. Graphite rod coated with/without Multi-walled Carbon Nano Tubules/ Poly aniline (MWCNT/ PANI) was used as anodic electrode. Microbial mixed culture was inoculated into the anodic chamber along with finely pasted and diluted agricultural waste. Phosphate buffer pH 7 was used in Cathodic chamber. The microbial fuel cell was allowed to run for one week under stable condition and then power generation in all successive organic waste degradation process and their COD removal was observed. A significant amount of reduction in COD was observed using MFC when 35% diluted substrate was used. Further, a maximum open circuit voltage of 222.7 mV was observed when MFC was operated with graphite felt electrode in anode. Vast enhancement in open circuit voltage was found when graphite felt electrode was coated with CNT/PANI and used as anode.

Keywords: Microbial Fuel cell (MFC), Cathode, Graphite felt, Chemical Oxygen Demand (COD).

Antibiotic Producing Actinomycetes Isolated From Various Geographical Regions Of Nepal

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Abstract:

Resistance to antibiotics is increasing everyday which can be solved by the production of novel antibiotics. Actinomycetes have been the primary sources of such compounds in the world. The primary goal of this study (October,2016- June,2017) was to study the antimicrobial potential of actinomycetes isolated from soil from different parts of Nepal.Using serial dilution-agar plating on starch casein agar, 111 actinomycetes were isolated from 27 soil samples. 39 isolates (35.1%) showed antimicrobial activity against at least one of 4 target organisms viz. *E. Coli* ATCC25922 (EC), *Klebsiella pneumonia* ATCC 700603 (KP), *Pseudomonas aeruginosa* ATCC 27853 (PA) and *Staphylococcus aureus* ATCC 25923 (SA) during initial screening using perpendicular streak method on nutrient agar. The genera of these active isolates were identified using coverslip culture based on the morphology of the aerial and substrate mycelia and spore chain. Among the 39, the most common was *Streptomyces* (61.5%) followed by *Dactylosporangium* (13%), *Nocardia* (5%), *Nocardiopsis* (5%), *Micromonospora* (3%), *Planomonospora* (3%), *Pseudonocardia* (2%) while the rest could not be identified. 8 most effective isolates were chosen arbitrarily based on spectrum of activity and underwent fermentation in Casein Starch Peptone Yeast Extract-Malt Extract broth. The crude antibiotic was prepared with solvent extraction using ethyl acetate and solubilized in phosphate buffer. Its effectiveness was tested using well-diffusion method on Mueller Hinton agar against the three Gram-negative organisms and an MRSA strain. 6 (5 *Streptomyces* and 1 *Micromonospora*) out of the 8 most effective isolates exhibited zones of inhibition against at least 1 isolate in the secondary screening. The most effective extract was obtained from isolate B2.7 (*Streptomyces* spp), the diameters of the zones of inhibition were: EC (22.7 ± 2.5 mm), KP (19.7 ± 0.6 mm), PA (21.7 ± 1.2 mm) and MRSA ($26 \text{ mm} \pm 1 \text{ mm}$). The minimum inhibitory concentration of this extract against EC was found to be 3.125 mg/ml, determined by using micro dilution technique with resazurin dye.

Keywords: Antibiotics, Actinomycetes, Minimum inhibitory concentration, Antimicrobial activity

Post Docking Analysis And Comparative Studies Of Drugs With BRCA1 And BRCA2.

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Abstract:

According to a survey conducted by USPS, approximately 12.4% will be diagnosed with breast cancer at some point during their life time. It has become the most common type of cancer amongst women and also causes over 8.2 million deaths (2012, WHO). With the advent of breast cancer, it is critical to develop drugs which help cure breast cancer. A comparative study of 4 drugs was carried out after docking with BRCA1 and BRCA2. iGEM-DOCK was used for docking and drug molecules and structures were obtained from Drug bank and PubChem. Sketching was carried out using chemsketch. A comparative analysis showed that the drugs used show better binding to BRCA1 receptor protein as compared to BRCA2 receptor protein. Final energy, hydrogen bonds and van der waals interactions indicate that the drugs bind to BRCA1 receptor protein far more effectively as compared to BRCA2 protein receptor. BRCA1 and BRCA2 structures were compared using UCSF chimera.

Keywords: BRCA1, BRCA2, Docking.

Multiple Shoot Propagation Of Paulownia Tomentosa By In-Vitro Layering (Stool-Shoot Method)

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Abstract:

Paulownia is a fast-growing, woody tree native to the forests of China. It belongs to the family Paulowniaceae (previously Scrophulariaceae) and is majorly used as a source of wood for furniture and musical instruments. In-vitro layering or 'stool shoots' is a technique to achieve multiple shoot propagation where shoots with several nodes are placed horizontally on the surface of nutrient medium. Lateral growing points of nodes form a thicket of small growing shoots. In-vitro layering (stool shoots) was performed on shoots of Paulownia tomentosa having one, two and three nodes to compare the amount and efficiency of multiple shoots produced on the nodes. The in-vitro layering was done in MS medium supplemented with 0.1 mg/l NAA and 2 mg/l BAP. For each layering method, 40 explants were cultured. The average shoot growth per node was found to be 1.42, 1.40 and 1.66 for explants with one, two and three nodes respectively.

Keywords: In-vitro layering, MS medium, Multiple shoot propagation, Paulownia tomentosa, Stool shoots

Synergystape: A New Tool For Effective But Less Toxic Synergy Scoring

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Abstract:

Identification synergistic drugs combinations is essential for treatment of drug resistant cancers along with other infectious diseases. There is pressing challenge to develop a scoring system that will allow weighting more for less toxic but effective drug combinations. Proper weighting of synergy matrix can be a weigh to detect synergistic drug combination with comparably less toxicity. Weighting the synergy matrix points by respective single drug concentrations is under practice. Here in this study, we have identified bias produced due to weighing by concentration terms during synergy scoring and suggested a new method for scoring synergy using instantaneous response of single drugs at different concentrations. We have also developed new open access tools that perform better than existing tools for synergy scoring.

Keywords: Drug combination, Synergy scoring, Inhibition level, Toxicities

Cellulolytic Activity Of Soil Streptomyces Species Isolated From Different Region Of Nepal

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Abstract:

Cellulose is the most abundant renewable carbon source available on the earth for production of commercial value added feedstock chemicals, when converted efficiently to monomeric D-glucose units. In industry, these enzymes have found novel applications in the production of fermentable sugars, ethanol, organic acids, detergents and other chemicals. Cellulase enzymes hydrolyze cellulose to form primary products glucose, or shorter polysaccharides and oligosaccharides. The cellulolytic activity of twenty eight strains of *Streptomyces* spp isolated from soil was analyzed. Out of twenty eight, eleven isolates showed cellulolytic activity which was confirmed with clear zone around the colony on carboxy methyl cellulose agar medium using congo red. The enzyme assays like filter paper cellulase (FPCase), and carboxy methyl cellulase (CMCase), were examined by methods recommended by the International Union of Pure and Applied Chemistry. Production of cellulase enzyme by a *Streptomyces* strain (4RO) was detected on cellulose agar (CA) medium after 4 days of incubation at 28°C that exhibited a clear zone of 20 mm around the colony. Cellulase production was assayed by measuring the amount of glucose liberated in $\mu\text{mol/ml/min}$ by using the dinitrosalicylic acid assay method.

Keywords: *Streptomyces*, Cellulase, Enzyme assay, FPCase, CMCase.

Pre-Induced Abiotic Stress: Prospects For Disease (Powdery Mildew) Resistance In Tomato Plants

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Abstract:

Fungicides – commonly used in plant disease control – are expensive; not only economically but environmentally too. It is one of the major sources of environment deterioration and evolution of new resistant strains. As an alternate, inducing the plants immune system at the early stage of plant growth with weak shocks of abiotic stress aiming disease resistance can be a novel approach, which is economical as well as eco-friendly. Tomato (Srijana hybrid) was used as model plant and to protect them from powdery mildew (*Oidium* spp. and *Leveillula* spp.), immune system, at an early seedling stage, was induced through application of drought and herbicide (glyphosate) stress. After 25 days of induction of abiotic stress, plants were inoculated with two doses of fungus: 104 conidia per ml, 106 conidia per ml for low and high dose respectively and the disease response was studied by using Area Under Disease Progression Curve (AUDPC) up to 27 days post inoculation (dpi). Hydrogen peroxide released by plants was quantified 20 days after abiotic stress induction (before the inoculation of powdery mildew fungus) and total phenolic content (TPC) at 7 days post inoculation (dpi). Both drought and herbicide treated plants showed elevated levels of hydrogen peroxide compared to that of control. Drought stressed plants also showed enhanced levels of phenolic compounds and less area under the AUDPC – and higher biomass compared to that of the control plants showing increased resistance to the fungus. This refers to the induction of phenolic compounds synthesis by drought and the anti-fungal effect of the phenolic compounds to enhance the resistance of the plants.

Keywords: AUDPC, Disease, Dpi, Pesticide, ROS, Stress, Tolerance

Isolation And Characterization Of Novel Lytic Bacteriophage Against Carbapenem Resistant Bacteria For Potential Use In Phage Therapy

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Abstract:

Carbapenem-resistant enterobacteriaceae are among the most prioritized critical pathogens according to WHO. Their emergence and spread has now become a major public health concern creating serious problem in treatment of infectious diseases. Carbapenem-resistant enterobacteriaceae includes *Klebsiella pneumoniae* producing KPC- type carbapenemase, *Escherichia coli* and *Pseudomonas aeruginosa* producing ESBL. The use of bacteriophages for killing bacteria has drawn recent attention, which has potential as alternative to antibiotics. The phages are referred as bio-control agent as they can kill multi-drug resistant strains in the environment. In this study, we aimed to isolate bacteriophage against carbapenemase- producing *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and characterize for potential phage therapy as an alternative to antibiotics.

Seven different strains from host bacteria (*K. pneumoniae* and *P. aeruginosa*) were collected identified with biochemical test and antibiotic susceptibility pattern. Molecular identification of bacterial isolates was confirmed by amplification of the 16srRNA, *blaKPC*, *blaNDM* and *blaOXA* gene. Lytic phages were isolated against those host strains through double-layer agar assay method. Phages were purified by successively sub-culturing single plaque & standard spot assay. Life cycle, biological features, multiple host range, sensitivity of phage to temperature and pH was determined. Transmission electron microscopy was done for morphological identification.

Fifteen lytic phages against carbapenem resistant bacteria (6 phages against *Pseudomonas aeruginosa* and 9 against *Klebsiella pneumoniae*) were isolated from water sample from three different places (Balkhu, Teku and Basundhara). Among these phages, one of the most potent bacteriophage against kpc producing *K. pneumoniae* was selected and characterized morphologically and physiochemically, which showed stability between temperature 0-70 °C and pH range 3-11. TEM image revealed that it belongs to Podoviridae. Among fifteen phages, twelve phages showed multiple host range spectrum within same genus while three were extensively specific. Our result showed that phages showed multiple host range as well as effectively killed multi-drug resistant bacteria which can possibly be used in therapeutics and as professed in scientific world.

Keywords: Bacteriophage; Carbapenem-resistance bacteria; *Klebsiella Pseudomonas*, TEM

Extraction, Isolation, Purification And Optimization Of Amylase And Protease Isolated From Bacillus Spp.

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Abstract:

Amylase and protease are abundantly present in nature. Their main source is the microbial origin. *Bacillus* species are an important source of fine biochemical, antibiotics and insecticides. It is known that about two-third of the industrial enzymes (amylase, protease, cellulose, penicillinase, chitinase, etc,) are produced by the *Bacillus* spp. Amylases are hydrolyzing enzyme in function which causes hydrolysis of molecules. Amylase hydrolyzes the α -1, 4-glucosidic bonds in starch and related polysaccharides. Two principal types of amylase are produced by the bacilli, the liquefying and saccharifying enzymes. Protease is a single class of enzyme which occupy a pivotal position due to their wide applications. Among bacteria, *Bacillus* species are specific producers of extracellular enzymes. The present work comprised of amylase and protease producing microorganisms. The study focused on the optimization of the microorganisms that are enzyme producers. To isolate and identify the amylase and protease producing strain, soil samples were collected from different vegetation at the altitude of 4367.35 feet. The isolates were screened and various biochemical tests and morphological observations were done to identify the isolates. The enzymes were produced by the submerged state fermentation (SmF) from the isolates and purified by dialysis. Effects of temperature, pH, and different carbon and nitrogen sources of the medium using SmF were optimized. Among 95 isolates, 36 were identified. Among the identified isolates, *Bacillus subtilis* and *Bacillus thuringiensis* were optimized for the amylase and protease production respectively. The maximum amylase production was found at 42°C temperature, in fructose as a carbon source, peptone as a nitrogen source and at pH 7. Similarly, the maximum protease production was found at 42°C temperature, in sucrose as carbon sugar, ammonium sulphate as a nitrogen source. The enzyme production by the optimized *Bacillus subtilis* and *Bacillus thuringiensis* at 42°C were found to be 42 U/ml/min and 52 U/ml/min respectively.

Keywords: Amylase, Protease, *Bacillus* spp, Extraction, Purification, Optimization

Initial Growth Analysis Of Bambusa Balcooa Roxb. And Bambusa Tulda Roxb. Growing In Agro Ecosystem Of Central Siwalik Region, Nepal.

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Abstract:

B. balcooa and *B. tulda* are very fast growing, highly productive and predominant species in Central Siwalik region. The growth pattern of bamboo was found to have two distinct periods, the first being culm height growth and the second which commences after attaining maximum height is the accumulation of dry mass and increase strength until complete maturation. The sprouting young culms of bamboo were tagged during June and July, 2016. Soil sample analysis from five different depth (0, 10, 20, 30 and 40 cm) of *B. balcooa* and *B. tulda* growing site for physico-chemical and microbial analysis showed the average result of moisture content (7.61; 7.02 %), electrical conductivity (1.04; 1.01 ds/m), extractable phosphorus (25.09; 23.08 kg/ha), pH (6.82; 5.92), bulk density (0.87; 1.39 gm/cm³) and water holding capacity (754; 683 ml/l) and microbial biomass from bamboo rhizosphere (767600; 695400 CFUs/gram) respectively. Thirty randomly selected culms of different dbh (diameter at breast height) classes for each species were carefully analyzed by non-destructive growth analysis method for various growth parameters for two times in an interval of three months. The growth analysis of *B. balcooa* and *B. tulda* recorded the mean value for culm length (18.12 ± 0.68 ; 17.34 ± 0.51 m), culm diameter (9.0 ± 0.31 ; 7.54 ± 0.08 cm), no. of branches (28.0 ± 6.92 ; 16 ± 1.51), branch length (60.19 ± 18.49 ; 34.75 ± 0.82 cm), no. of nodes (120.80 ± 0.65 ; 19.30 ± 0.63), length of internode (5.41 ± 0.25 ; 4.41 ± 0.06 cm) and specific leaf area (40.93 ± 1.32 ; 38.42 ± 1.25 cm²) respectively. The correlation analysis of culm diameter and culm height of *B. balcooa* revealed strongly positive relation ($r = 0.90$) whereas *B. tulda* exhibited less positive correlation ($r = 0.70$). This study revealed that the *B. balcooa* has better growth performance than *B. tulda* despite the fact that physico-chemical parameters of soil have non-significant differences. *B. balcooa* deserves high prospect for its application in pulp and paper industry, raw material for construction, soil rehabilitation, etc. however, it demands further studies in more detail.

Keywords: Agro ecosystem, Bamboo, Correlation Analysis, Growth Analysis, Siwalik Region

Biofilm Detection And Antibiotic Sensitivity Patterns Of Uropathogens In Patients With Indwelling Urinary Catheter In A Tertiary Care Hospital In Nepal

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Abstract:

Background: Catheter-Associated Urinary Tract Infection (CAUTI) is a common health care associated infection worldwide. The resistance mechanisms are usually based on the delayed penetration of the antimicrobial agent in to the biofilm, changes in the microbial growth rate or other physiological alterations related to the development of the biofilm and its early detection prevents various risks and economic burden. This study was aim to isolate uropathogens in catheterized patients, their antimicrobial susceptibility pattern, rate of biofilm production and correlate the biofilm production with antimicrobial susceptibility pattern.

Among 105 samples, 65 were found to be positive for uropathogens. *Escherichia coli* were found to be most frequent isolates accounting 57%, followed by *Klebsiella pneumoniae* 15%. The study revealed that 46% isolates were biofilm producers. More biofilm producers were found to be *E. coli* 33%, followed by *Klebsiella pneumoniae* 30%. Higher antibiotic resistance was observed in biofilm producers than in biofilm non-producers. The antibiotic susceptibility pattern in this study shown Ampicillin & Amoxicillin-clavulanate were least active drug, whereas Piperacillin-tazobactam & Imipenem were most effective drugs among gram negative isolates. Among Gram positive isolates Amoxicillin-clavulanate & Tetracycline were least active, whereas Vancomycin & Meropenem were most effective antibiotic. It can be concluded that *Escherichia coli* was most frequent isolates and more biofilm producer which showed higher sensitivity to Piperacillin-tazobactam & Imipenem. Correlation was observed between biofilm production and multidrug resistance.

Keywords: Biofilm, Uropathogens, Multidrug resistant

Drug And Shrna Library-Based Screening For Therapeutic Targets Of Myocardial Ischemia-Reperfusion Injury

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Abstract:

Ischemic heart disease is the leading cause of death in the Western countries. In case of an acute myocardial infarction, the most dramatic manifestation of the disease, reperfusion of the occluded coronary artery is inevitable. However, reperfusion partly exacerbates the injury by several mechanisms, causing so called ischemia-reperfusion (I/R) injury. Different cardio protective drug therapies have been proposed to alleviate the injury and to improve regeneration of myocardium. For the time being, their translation into clinical use has not been successful. To search for novel therapeutic targets of the I/R injury, we performed screenings on HL-1 cardiomyocytes using an shRNA library, consisting of 25,000 shRNAs targeting 4,625 genes, and a drug library consisting of 694 clinically used drugs. To model I/R injury, cells were exposed to hypoxia-reoxygenation (H/R) treatment or grown in normoxia as controls. Relative shRNA enrichment and depletion in the H/R-treated cells, as compared to normoxic cells, was measured after sequencing of the shRNA-specific barcodes from the cell populations. For drug screening, cell viability was used as the read-out. These two screening approaches were combined for pathway analysis using dimensionality reduction. The most important signaling pathways and their representative members, most notably EGFR and PRKACA, were selected for further validations in two cardiomyocyte cell lines.

Keywords: Myocardial ischemia, Cardiomyocyte, Hypoxia, Drug screen, shRNA screen

Comparison Of Nasal Colonization Of Methicillin Resistance Staphylococcus Aureus (MRSA) In HIV And Non-HIV Patients Attending National Public Health Laboratory, Teku, Kathmandu

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Abstract:

Staphylococcus aureus is a major source of community and hospital acquired infections. Methicillin resistant Staphylococcus aureus (MRSA) with intrinsically developed antimicrobial resistance has been associated with increasing morbidity and mortality of the patients. Increasing, incidence of non-beta-lactam antibiotics has become possible threat in treatment of S. aureus infections. Nasal carriage of

S. aureus plays a vital role in spread of endogenous infections. This study was conducted from June 2016 to December, 2016 at National Public Health Laboratory (NPHL), Kathmandu with aim to assess the rate of S. aureus nasal carriage and MRSA carriage. A total of 600 nasal swabs, 300 each from HIV and non-HIV patients were processed by culture technique and antibiotic susceptibility test (AST) was performed by disc diffusion method. The S. aureus nasal carriage among HIV was found to be 80(26.66%) while in non-HIV was 45(15%), MRSA carriage in HIV was 11(3.6%) and non-HIV was 3(1%).The more carrier were HIV males 40(26.49%) while MRSA was most among HIV females 7(5.07%). The highest carriage was among 31-40 years 36(45%) of HIV patients while highest carriage in non-HIV was in 21-30 age groups with 13(28.9%). The association of S. aureus carriage was statistically significant in HIV patients (p – value < 0.05) and probable cause for this may be due to immune suppression and frequent exposure to hospital settings. Thus, regular surveillance and monitoring of carriage and antimicrobials use is foremost for S. aureus infections control.

Keywords: Nasal Carriage, MRSA, HIV, NPHL, AST.

Predictive Modeling Of Binding Affinities Between Chemical Compounds And Protein Targets For Drug Discovery And Repurposing Applications

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Abstract:

Due to relatively high costs and labor required for experimental profiling of the full target space of chemical compounds, various machine learning models have been proposed as cost-effective means to advance this process in terms of predicting the most potent compound-target interactions for subsequent verification. However, most of the model predictions lack direct experimental validation in the laboratory, making their practical benefits for drug discovery or repurposing applications largely unknown. We therefore introduced and carefully tested a systematic computational-experimental framework for the prediction and pre-clinical verification of drug-target interactions using a kernel-based regression algorithm as the prediction model. To evaluate its performance, we first predicted unmeasured binding affinities in a large-scale kinase inhibitor profiling study, and then experimentally tested 100 compounds - kinase pairs. The relatively high correlation of 0.77 between the predicted and measured bioactivities supports the potential of the model for filling the experimental gaps in existing compound-target interaction maps. Further, we subjected the model to a more challenging task of predicting target interactions for such a new candidate drug compound that lacks prior binding profile information.

As a specific case study, we used tivozanib, an investigational VEGF receptor inhibitor with currently unknown off-target profile. Among 7 kinases with high predicted affinity, we experimentally validated 4 new off-targets of tivozanib, namely the Src-family kinases FRK and FYN A, the non-receptor tyrosine kinase ABL1, and the serine/threonine kinase SLK. Our sub-sequent experimental validation protocol effectively avoids any possible information leakage between the training and validation data, and therefore enables rigorous model validation for practical applications. These results demonstrate that the kernel-based modeling approach offers practical benefits for probing novel insights into the mode of action of investigational compounds, and for the identification of new target selectivities for drug repurposing applications.

Keywords: Drug - target interaction, Kinase, Kinase inhibitor, Machine learning, Drug discovery, Drug repurposing, Experimental validation

Xylanase Production By Solid State Fermentation From Candida Species And Testing Its Genotoxicity

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Abstract:

In recent years, increasing concern over preserving resources and environment has initiated a growing interest in producing microbial enzymes. Xylanase from microorganisms have attracted a great deal of attention in the last decade because of their biotechnological potential in various industrial processes such as waste water treatment, food, feed, and paper-pulp industries. The production of xylanase from *Candida* strain was investigated. The fermentation conditions were standardized for the growth and enzyme activity, the optimum being 72 – 96 hrs growth at initial pH 4.0, and cultivation temperature at 35°C. As carbon sources on xylanase production, corn husk was used and the highest yields xylanase were obtained. Genotoxicity testing by AMES test using *Salmonella typhimurium*, predicts the potentiality of xylanase enzyme to cause mutation or not. This study suggests that corn husk could be utilized as a carbon source for economical production of xylanases by *Candida* sp., and the results of AMES test proves the enzyme is non mutagenic. Thus, in turn reduce the cost of enzyme production leading to efficient use of ligno-cellulosic materials to produce value-added products.

Keywords: Xylanases, Genotoxicity, Solid State Fermentation, Non Mutagenic, Ames Test

Patient-Customized Drug Combination Prediction And Testing For T-Cell Prolymphocytic Leukemia Patients

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Abstract:

The molecular pathways that drive cancer progression and treatment resistance are highly redundant and variable between individual patients with the same cancer type. To tackle this complex rewiring of pathway cross-talks, personalized combination treatments targeting multiple cancer growth and survival pathways are required. We implemented a computational-experimental strategy, drug combination prediction and testing (DCPT) platform, for efficient in silico prioritization and ex vivo testing in patient-derived samples to identify customized synergistic combinations for individual cancer patients. DCPT uses drug-target interaction networks to traverse across the massive combinatorial search spaces among 218 compounds (a total of 23,653 pairwise combinations), and identifies cancer-selective synergies by using differential single-compound sensitivity profiles between the patient cells and healthy controls, hence reducing the likelihood of toxic combination effects. A polypharmacology-based machine learning modelling and network visualization makes use of baseline genomic and molecular profiles to guide patient-specific combination testing and clinical translation phases. Using T-cell prolymphocytic leukemia (T-PLL) as a first case study, we show how the DCPT platform successfully predicted distinct synergistic combination for each of the three T-PLL patients, each representing with different resistance patterns and synergy mechanisms. In total, 10/24 (42%) of the selective combination predictions were experimentally confirmed to show synergy in patient-derived samples ex vivo.

Keywords: Drug combinations, Synergistic effects, Machine learning, Ex-vivo testing, T- cell prolymphocytic Leukemia

Immuno-Molecular Epidemiological Analysis Of Dengue Virus Circulating In Nepal.

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Abstract:

The emergence and circulation of viral infection has become one of the major public health concerns in the world. Of those emerging diseases, Dengue is a mosquito borne flavivirus mainly prevalent in the tropical and sub-tropical countries of the world. Four serotypes of dengue virus (DENV 1- 4) are globally present. Hundreds of Dengue cases are reported annually and the re-emergence of this virus with different serotypes has raised the concern of public health in Nepal. No advanced methods besides the Rapid Diagnostic kits are applied for diagnosis in most of the hospitals in Nepal. Rapid tests are not reliable as this coincides with the similar flavivirus infections. IgM, IgG and NS1 ELISA tests are the preliminary tests while RT-PCR is the confirmatory test for Dengue infection. The detection of dengue virus infections has great importance for the clinical management of patients, surveillance, and clinical trial assessments in the days to come.

240 Dengue suspected clinical samples were subjected to Dengue IgM, IgG and NS1 capture ELISA followed by Viral RNA extraction. cDNA was prepared, and RT-PCR was performed using D1 and DencomR2 primers. Dengue Serotyping was then done using gene specific primers. Out of 240 acute dengue cases, 64 % of them were male and 2% of them were below age of 10. About 60% were NS1 positive, 33% were IgM positive and 7% were IgG positive. Most of the cases were of primary infection. About 37% of them were confirmed positive by PCR. Dengue Serotype-1 was found to be prevalent in the year 2016 in Nepal. Only 37% of the clinically suspected cases were confirmed for Dengue virus infection. This implies that confirmatory tests like PCR should be made for the proper diagnosis of the disease and medication to be followed accordingly. To add, ELISA is the preliminary test and RT-PCR is the confirmatory tests for the detection of Dengue virus.

Keywords: Dengue virus, RT-PCR, ELISA, Re-emergence, Serotyping



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Investigation Of Altitudinal Effect On Secondary Metabolites Of Different Medically Important Plant Species Of Nepal

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Abstract:

Plants have been important sources of human medicines since the beginning of human civilization. Many herbal remedies have been employed in various medicinal systems for treatment and management of different chronic and infectious diseases. Nepal has diverse geographic locations ranging from 60m to 8848m above sea level. A lot of medically important plants are found in this wide range of altitude which have varying bioactivities. Our research was focussed on collecting different medically important plant species from different altitudes of Nepal and comparing their bioactivities (antibacterial, antioxidant and cytotoxic properties). We have collected altogether seven medically important plant species (*Camellia sinensis*, *Curcuma longa*, *Cuscuta reflexa*, *Moringa oleifera*, *Myrica esculenta*, *Psidium guajava* and *Punica granatum*) from two different altitudes of Nepal. The crude methanolic extracts of these plant species were prepared and their antibacterial, antioxidant and cytotoxicity activities were assessed.

Almost all the plant extracts have shown antibacterial property against gram positive bacteria except *Moringa oleifera* while neither of the plants showed antibacterial property against Gram negative bacteria. The highest zone of inhibition was shown by *Punica granatum* collected from Hattiban against *Staphylococcus aureus* with diameter of 1.7cm. The strongest antioxidant activity was shown by methanolic extract of *Punica granatum* collected from Janakpur with IC₅₀ of 5.52µg/ml whereas the lowest antioxidant activity was shown by methanolic extract of *Moringa oleifera* collected from Janakpur with IC₅₀ of 593.33µg/ml. Among all the plant species, the high altitude plants showed higher antioxidant activity with *Punica granatum* being the exception. Likewise all the plants were found to be highly toxic with LC₅₀ < 100µg/ml except *Psidium guajava* which showed medium toxicity with LC₅₀ > 100µg/ml. The lowest LC₅₀ was shown by *Curcuma longa* collected from Syangja (LC₅₀= 10.07µg/ml) and the highest LC₅₀ was shown by *Psidium guajava* collected from Janakpur (LC₅₀= 141.38µg/ml). It was found that the higher altitude plants showed higher LC₅₀ than the lower altitude plants with *Punica granatum* being only the exception.

Keywords: Altitude, Medicinal Plants, Antibacterial, Antioxidant, Toxicity, IC₅₀, LC₅₀, Zone of Inhibition

Molecular Confirmation Of Leishmaniadonovani Parasites In Non –Program Districts Of Nepal

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Abstract:

The intracellular protozoan parasite, Leishmania is responsible for the disease termed Leishmaniasis which has been reported from 98 countries around the world. Among various forms of Leishmaniasis, the most common in the Indian sub-continent is Visceral Leishmaniasis, commonly called kala-azar. It was previously endemic in the Terai region of Nepal particularly in 12 districts of the southeast region adjoining to Bihar state (in India). These districts have been designated as program districts by the government of Nepal. For this study, 16 suspected cases of visceral leishmaniasis patient blood samples were collected from the non-program districts of Nepal. The blood samples were then used for DNA extraction of the protozoan and subjected to polymerase chain reaction for amplification using primers specific for the variable region of the kinetoplastminicircles. An amplicon size corresponding to the band length of approximately 720 base pairs confirmed the presence of the parasite in five of the blood samples. When tallied with the positive control, the parasites were confirmed as Leishmaniadonovani. Of particular significance among the confirmed cases was that of patient residing in Dolpa. This case in particular is probably the first case report for the detection of parasite at altitudes above 5000 m above sea level. Although the number of cases for leishmaniasis is on the decline, the infectious disease however is progressively spreading towards the non-endemic parts of Nepal which presents a significant hurdle for the government that aims on eliminating the cases by 2020.

Keywords: Kinetoplast, Leishmaniasis, Leishmaniadonovani, Non-Program Districts, Nepal

An Approach To Treat Dairy Waste In Microbial Fuel Cell

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Abstract:

This work studied the feasibility of bioelectricity generation and dairy waste treatment using a two chamber electron mediated Microbial Fuel Cell (MFC) fabricated with graphite felt as an electrode and paneer whey as anodic substrate. 0.1M phosphate buffer was used as cathodic solution. Result showed that the treatment of waste in MFC found to reduce total sugar by 95.06%, phosphorous by 65.67%, ammonical nitrogen by 67.13% and COD by 58.27% indicating the efficiency of MFC in removal of pollutants and waste water treatment. Mixed culture of bacteria and lactobacillus spp showed the maximum open circuit voltage of 528.4mV and power of 13.510W/m³ on the fifth day using external resistor of 1000Ω; in comparison to single strain of Klebsiella spp and Lactobacillus spp. This indicates that the electrogenic mixed culture of bacteria are more electrochemically active than the single culture bacteria in MFC. When 0.1M of potassium ferricyanide (2ml/min) was added to the phosphate buffer the MFC showed the maximum OCV of 609.5mV and maximum power of 22.261W/m³ on the fourth day using the 1000Ω resistor.

The graphite electrodes coated with MWCNT composite treated with absolute ethanol showed the maximum power of 25.869W/m³ with 1000Ω resistor on the fifth day using the phosphate buffer enriched with potassium ferricyanide.

Keywords: Dairy waste, Microbial fuel, Klebsiella spp, Lactobacillus spp

PCR Based Diagnosis And DNA Barcoding Of *Candidatus liberibacter* Bacteria Associated With Huanglongbing Disease In The Central Nepal

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Abstract:

Citrus production continues to decline in Nepal particularly due to Citrus greening disease also called as Huanglongbing (HLB) disease. HLB disease is caused by a vector-transmitted, phloem restricted bacteria *Candidatus Liberibacter* spp. It is a gram-negative fastidious bacterium which is impossible to culture in artificial medium that makes its diagnosis more complicated. Leaves and fruits of HLB affected plants show VISUAL symptoms that resemble to other disorders like micronutrient deficiencies, such as zinc, manganese and iron. In this context quick and reliable polymerase chain reaction (PCR)-based molecular diagnosis has commonly been used globally for rapid detection and identification. DNA was isolated from the mid rib pieces taken from suspected leaves samples using Qiagen DNeasy plant minikit. Conventional PCR were performed using DNA and specific primers that target 16s ribosomal DNA (rDNA) genes for confirmation of disease. Out of 37 different suspected citrus samples collected from citrus pocket areas of Kaski, Gorkha, Tanahun, and Lamjung districts of Nepal, 27 gave positive result for HLB. Three positive samples from Lamjung (two positive samples) and Tanahun districts were sequenced. Sequence analysis showed similarity with *Candidatus Liberibacter asiaticus*. This research work focuses on the importance of PCR-based diagnosis and DNA barcoding for the integrated management of Huanlongbing disease in Nepal.

Keywords: 16s rDNA, *Candidatus Liberibacter africanum*, *Candidatus Liberibacter asiaticus*, *Diaphorina citri*

Xylanase Production By Solid State Fermentation From Candida Species And Testing Its Genotoxicity

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Abstract:

In recent years, increasing concern over preserving resources and environment has initiated a growing interest in producing microbial enzymes. Xylanase from microorganisms have attracted a great deal of attention in the last decade because of their biotechnological potential in various industrial processes such as waste water treatment, food, feed, and paper-pulp industries. The production of xylanase from *Candida* strain was investigated. The fermentation conditions were standardized for the growth and enzyme activity, the optimum being 72 – 96 hrs growth at initial pH 4.0, and cultivation temperature at 35°C. As carbon sources on xylanase production, corn husk was used and the highest yields xylanase were obtained. Genotoxicity testing by AMES test using *Salmonella typhimurium*, predicts the potentiality of xylanase enzyme to cause mutation or not. This study suggests that corn husk could be utilized as a carbon source for economical production of xylanases by *Candida* sp., and the results of AMES test proves the enzyme is non mutagenic. Thus, in turn reduce the cost of enzyme production leading to efficient use of ligno-cellulosic materials to produce value-added products.

Keywords: Xylanases, Genotoxicity, Solid State Fermentation, Non Mutagenic, Ames Test

Study Of Cellulolytic Fungi Isolated From Soil And Compost Samples Of Kathmandu Valley

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Abstract:

The use of cellulase in various industries is increasing day by day so its demand is also increasing. Hence, it is necessary to screen and identify new strains exhibiting efficient cellulase production. In this study, 20 soil samples and 20 compost samples were collected from different areas of Kathmandu valley. Fungi were isolated and cellulase positive isolates were identified by standard microbiological techniques. Enzyme was produced from isolates showing highest zone of hydrolysis and purified partially by acetone precipitation method. The activity of enzyme from both isolates at different pH, temperature and substrate concentration was studied.

A total of 83 and 91 fungal isolates were obtained from soil and compost samples respectively. Among them, 12 isolates from soil and 15 from compost showed cellulase positive activity. Among all the positive isolates, *Penicillium* spp. from soil and *Trichoderma* spp. from compost samples showed the highest cellulolytic activity. Enzyme produced by *Penicillium* spp. had an optimum pH of 6 while that of *Trichoderma* spp. had an optimum pH of 5. The optimum temperature for enzyme produced by both isolates was 28°-37°C but enzyme from *Trichoderma* spp. was found to be active up to 70°C while that of *Penicillium* spp. was active only up to 60°C. The activity of both enzymes was highest at 1% carboxymethyl cellulose. The activity of *Trichoderma* spp. was significantly higher ($P<0.05$) at all conditions than that of *Penicillium* spp. Also, there was a significant difference ($P<0.05$) between crude and partially purified enzyme.

Keywords: Cellulase, *Penicillium* spp., *Trichoderma* spp., Enzyme Activity

DNA Barcoding Of Medicinal Plants From Central Region Of Nepal

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Abstract:

DNA barcoding is a molecular tool that uses short gene sequences taken from a standardized portion of the genome for species identification. It is entering a new phase of applications to address questions relating to taxonomy, ecology, evolution, and conservation of wildlife. Medicinal plants contain high value ingredients, which are being used in drug development pharmacopoeia, non- pharmacopoeia or synthetic drugs. In this study, 70 medicinal plants were collected from Mardi-Himal. Fresh leaves of economically important and endangered plants were collected and preserved in silica gel. Plant DNA was extracted using widely used CTAB protocol and Qiagen kits. Both chloroplast (rbcL) and nuclear markers (ITS) were used for PCR optimization. The amplification success rate for rbcL and ITS markers were found to be 87.2% (61) and 85.7% (60) respectively. High quality purified PCR products were used for sequencing. Sequence analysis of both markers have been identified and confirmed taxonomic identities of 67.14 % (47) plant samples. The research highlights the use of molecular markers in species identification; identify adulteration, authentication of herbal products and sustainable utilization and conservation of medicinal plants in Nepal.

Keywords: DNA Barcoding, Medicinal Plants, Molecular Markers, PCR Optimization, Sequencing

Screening And Characterization Of Myxobacteria For Production Of Novel Anti-Microbial Compounds

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Abstract:

Antimicrobial resistance (AMR) is an increasingly serious threat to global public health. Solution to this problem is very difficult as either new or novel compound has to be discovered or completely new way to deal with those diseases has to be developed keeping side effects at minimal. Myxobacteria is a delta gram negative gliding proteobacteria. More than 7500 different strains have been isolated among which yielded at least 100 natural products and 500 derivatives.

In this study, Myxobacteria was isolated, identified through morphological analysis of fruiting body and characterized for production of novel antimicrobial compounds. Various samples were collected from different places of Nepal and were then subjected to selective media for the isolation of different Myxobacteria. Myxobacteria capable of producing effective secondary metabolites were inoculated on broth for the first extraction which was then followed by ethyl acetate organic phase extraction. The extract was then checked against different potential pathogenic bacteria to compare its effectiveness against common antibiotics used.

The extract showed comparable inhibition zone against E.coli, Klebsiella and Pseudomonas. The inhibitory effect of Myxobacteria broth extracts was observed even in mild concentration. Since Myxobacteria is a non-pathogenic bacterium and has showed antibacterial activity, its property can be used to fight against multidrug resistant bacteria. It is hoped that this study will help to identify the novel compounds secreted by the myxobacteria which are native to Nepal itself.

Keywords: AMR, Myxobacteria, Novel antimicrobial compounds

Screening Of Carbohydrates And Lipids In Sugary Drinks And Dairy Products Using Paper Assay Methods

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Abstract:

Carbohydrate and Lipid assays are routinely performed in many research and clinical fields. In this research, we used paper-based carbohydrate and lipid assays for screening the levels of carbohydrates in wine and soft drink samples and total lipids in dairy products. The carbohydrate was measured using Anthrone-sulfuric acid colorimetric assay on paper platform and the total lipid was measured using Sulpho-phospho vanillin colorimetric assay on paper platform. Paper device contained a test zone where reaction was allowed by adding standard/sample and reagent. Smartphone was used to capture an image of paper after reaction and the image was analyzed using ImageJ to measure the intensity and ultimately find the concentration of analyte. We measured total carbohydrates in 19 Nepali wines and 24 soft drinks sample. Similarly total lipids were measured in 13 dairy samples collected from local marts in Kathmandu valley. The total carbohydrate in wines and soft drinks and total lipids in dairy samples ranged from 0.36 to 78.67 mg/ml (mean=28.32±26.20 mg/ml), 0.25 mg/ml to 253.12 mg/ml (mean=167.86±63.85 mg/ml) and 2.11 mg/ml to 15.68 mg/ml (mean=6.56±5.08 mg/ml), respectively. We compared the measured value with the results obtained using conventional (spectrophotometric) method. We found that the two methods had very strong positive correlation ($r^2 > 0.8$). The Bland and Altman Analysis of carbohydrate results in wines and soft drinks showed a negative bias of paper device with spectrophotometer (-24.51 mg/ml; 95% limits of agreement, -48.93 to -0.09; n=19 and -18.63 mg/ml; 95% limits of agreement, -39.69 to 2.43; n=24, respectively) and that of lipid results showed a good agreement with a positive bias of paper device with spectrophotometer (0.69 mg/ml; 95% limits of agreement, -0.93 to 2.31; n=13). The results of the assay (B&A analysis and $p < 0.05$) provides evidence for the potential of paper devices for quantitative analyses. We demonstrate that these paper-based assays may provide inexpensive alternative to quantify carbohydrates and lipids as it does not require expensive equipment, trained personnel, and require very less (in μ l) volume of reagent/sample.

Keywords: Paper based assay, Colorimetry, Anthrone-sulfuric acid assay, Sulpho-phospho vanillin assay

Green Synthesis Of Silver Nanoparticles And Their Antimicrobial, UV-Vis Spectroscopic And FTIR Analysis

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Abstract:

Nanoparticles are an ultra – microscopic particle that has at least one dimension less than 100 nm. Research into nanoparticle is currently gaining an immense interest due to its wide variety of potential applications in biomedical, optical, and electronic fields. Different chemical and physical methods have been employed for preparation of nanoparticle but there is a need for “green chemistry”. Green synthesis of nanoparticles provides cost effective, time saving, environmentally sound and safer method for nanoparticle synthesis. To this end, silver nanoparticles have been prepared by using aqueous solution of the selected medicinal plants viz. *Acorus calamus* (Bojo), *Canna indica* (Sarbadaphool), *Centella asiatica* (Gotu kola), *Colocasia esculenta* (Karkalo), *Ficus religiosa* (peepal), *Juglans regia* (Walnut), *Melia azedarach* (Bakaino), *Rhododendron lepidoteum* (Sunpati), and *Urtica dioica* (Sissno). The formation of AgNPs was first screened by measuring the surface plasmon resonance peak in the range of 380-440 nm using UV-vis spectroscopy. After preliminary analysis, further analysis was done using FTIR. The nanoparticles were then used for antimicrobial assessment using standard pathogenic strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* with Streptomycin antibiotic as standard. Among the tested nanoparticles, those produced from *Acorus calamus*, *Canna indica*, *Colocasia esculenta*, *Ficus religiosa*, *Juglans regia* and *Melia azedarach* showed the broadest antimicrobial activity inhibiting all the tested microbes. Similarly, the aqueous extracts of these plants did not show any antimicrobial activity indicating that antimicrobial activity was solely due to the nanoparticles produced. Hence, the synthesis of nanoparticles using these plants presents a promising aspect for generation of antimicrobial complexes.

Keywords: Antimicrobial, FTIR Analysis, Green Synthesis, Silver Nanoparticles

Characterization Of Clinically Important Mutants Of Human Secretagogin

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Abstract:

Secretagogin is a recently discovered hexa EF-hand family protein with its abundance in pancreatic, neuronal and neuro-endocrinal cells. It has multiple functions based on its presence at different cellular locations including cytoplasm, nucleus, and endoplasmic reticulum. Secretagogin is already identified as the biomarker of various cancers, tumors and is found to be associated with diabetes. This protein shows different properties in oxidized and reduced conditions which can be linked to its differential function in these environments. Many mutations in this gene are enlisted in database which are rare, related to pathologies and are not well studied. "Characterization of clinically important mutants of human Secretagogin" is an approach to improve our understanding regarding the consequences of such mutations on the structure and functions of the protein. For the same purpose, wild type human secretagogin along with its three mutants A216V, R77H and V108M were cloned in pET-21b vector. Wild type as well as mutant proteins were then overexpressed, purified and characterized using fluorescence spectroscopy, circular dichroism, isothermal titration calorimetry, analytical gel filtration chromatography, partial trypsin digestion and protein-protein interaction. The mutants showed altered structural and functional properties (interaction with insulin) than the wild type Secretagogin. Wild type of Secretagogin existed predominantly as monomer whereas all the mutants existed predominantly as dimer. Apo form of R77H mutant was least stable ($C_{1/2}$ 2.46M) while that of A216V mutant was equally stable to the wild type of protein ($C_{1/2}$ 2.65M) as determined by Guanidium hydrochloride unfolding assay. The apo forms of all the proteins were less stable than their holo forms except that of V108M whose apo ($C_{1/2}$ 2.66M) and holo ($C_{1/2}$ 2.68M) forms were almost equally stable. The ability to stably interact with insulin seemed to have been lost in all the mutants. Though the calcium binding property in mutants was not completely destroyed, there was some alteration in the affinity and mechanism for binding calcium. The mutant V108M showed the behavior completely divergent than the wild type of Secretagogin by virtue of which this mutation is believed to have most distressing biological consequences.

Keywords: Protein, Secretagogin, Calcium, Mutation, Apo (Calcium bound form), Holo (calcium free form), Oxidized, Reduced.

Determination Of Total Phenolic And Flavonoid Contents, Antioxidant And Antibacterial Activity Of 5 Selected Lichens From Sagarmatha National Park

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Abstract:

Lichens are the symbiotic organisms which consist of fungal partner (mycobiont) in association with algae (photobiont). The bioavailability of lichen is diverse which are found in high mountains to desert as well and some can make stones and trees as their substratum. Lichens are rich in phenolic compounds. The major secondary metabolites found in lichens are dibenzofuran (eg; Usnic acid), depsides (eg; gyphoric acid), depsidones (norstictic acid), xanthoness and terpenes derivatives containing phenolic groups which are responsible for the bioactivity shown by lichens.

The main purpose of present work was to study antioxidant and antibacterial activities along with the total phenolic and flavonoid content of methanolic extracts of the 5 lichens from Sagarmatha National Park. Total phenolic content was determined by FolinCiocalteu method and total flavonoid was determined by AlCl₃ method. Antioxidant activity was determined by DPPH free radical assay along with the determination of IC₅₀ value of each lichen. *Lobariaretigera* contained high amount of phenols (90.40mg/g dry extract) followed by *Lobariaretigera* (72.83mg/g dry extract). Similarly flavonoid content was also high in *L. retigera* (846.67mg/g dry extract) followed by *L. japonica* (572.67mg/g dry extract). Other three lichens *Heterodermialeucomella*, *Heterodermiaspeciosa* and *Ramalina* contained comparatively less amount of phenols and flavonoids. Highest antioxidant activity was also shown by *L. retigera* (IC₅₀=19.72 ±1.21 µg/ml) followed by *L. japonica* (IC₅₀=31.83 ±1.03 µg/ml). Antibacterial activity was assayed by determining Minimum Inhibitory Concentration. The MIC of methanolic extract of all lichens assayed for *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), *Enterobacter faecalis* (ATCC29212), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC700603) and *Salmonella typhi* (Clinical isolate) ranged from 0.78-25 mg/ml. According to the result obtained, *L. japonica* and *H. leucomela* were found to be most effective among all lichens and *E. coli*, *S. aureus* and *B. subtilis* were three highly susceptible microorganisms compared to other tested organisms.

Keywords: Lichens, Antioxidant, Antibacterial, IC₅₀, DPPH, MIC, Phenolics, Flavonoids

Thutocycle: Nepal's First Ever Cigarettes Waste Management And Recycling Initiative

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Abstract:

According to the World Health Organization, over a quarter of Nepal's 26 million people smoke regularly killing more than 15,700 people by tobacco caused disease, but the risks associated with the wastes is even more severe and unexplored. Every year 1.5 billion cigarettes are produced and butts generated from them end up into dumping site or open spaces as toxic trash. These butts contain remnants of tobacco, paper and filter which may contain around 4000 toxic chemicals and trace amount of metals present in cigarette. The filters used in cigarette are generally cellulose acetate, a kind of natural plastic which takes about 24 months to 10 years or more to degrade and the leachates are of serious concern. Reports suggest that single cigarette butt can contaminate 1 liter of water affecting the entire aquatic lives there. Various pollutions and events of toxicity due to butts inhaling have been observed. In addition, the waste generated during manufacturing process is another concern for industries.

In developed nations, already strategic waste management techniques have been adopted to manage all the cigarette related wastes. However in case of Nepal, even actual facts are unavailable regarding the cigarette wastes. Hence Thutocycle is Nepal's first ever initiative to find recycling solutions to every type of cigarette associated wastes that includes tobacco dust generated from cigarette industries and cigarette butts thrown after use. Thutocycle aims to drag the people's attention to the urgency for management of cigarette waste. Our preliminary data shows approximately 583 cigarette butts littered per km per day in a small area in Kathmandu. This is an underestimated value but still if we compare this for entire nation and throughout the year it is going to be a huge amount. So, through this project we collect these wastes, separate them into degradable and non degradable parts, biologically, physically and chemically treat them in the most affordable and eco-friendly manner and convert them into products that would be salable. Further applicability of these recycled products are being investigated as per the feasibility Hence we envision environment management, income generation and employment creation through our project.

Keywords: Cigarette butts, Filter, Environment, Recycling

Molecular Investigation Of Carbapenem Resistant Pathogens Prioritized As Critical By World Health Organisation

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Abstract:

Carbapenem are potent broad-spectrum beta lactam antibiotics that are used to treat infections caused by such pathogens particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. These multidrug resistant bacteria are categorized as critical group of pathogens by WHO. Carbapenem being last resort of drug; however development of resistance against it has now become global health concern. These pathogens exhibit resistance through various mechanisms. Most common being intrinsic mechanism, includes loss of porin, overexpression of efflux pumps system and AmpC whereas extrinsic mechanism includes carbapenemase gene/beta-lactamase gene (*bla*GES, *bla*KPC, *bla*IMP, *bla*SPM, *bla*VIM and *bla*NDM) and acquisition of resistance genes such as extended spectrum beta lactamase ESBL (*bla*SHV, *bla*TEM, *bla*VEB, *bla*PER and *bla*OXA types). In *Pseudomonas aeruginosa*, it was found that those outer membrane porins were lost or altered readily resist imipenem (a type of carbapenem) causing poor penetration. Similarly, meropenem, another type of carbapenem, are resisted due to active efflux pump system. AmpC and other beta lactamase hydrolyzes the antibiotics confers carbapenem resistance. In context of Nepal, limited studies have been carried out at molecular level for the detection of multidrug resistance in clinical isolates. Therefore, through this study we would like to further elaborate our understanding in different genes and protein involved in resistance which might have more potent role in developing resistant against carbapenem with the hope to provide effective measures to control multidrug resistant organism prevailing in our community

In this study antimicrobial susceptibility profile based on the disk-diffusion tests and molecular identification of carbapenem resistance gene of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was done by PCR method followed by sequencing. Out of 95 samples of *Pseudomonas*, 31.57% were ampC positive and 6.3% showed nDm positive in which 31.57% showed presence of *oprD* gene. Out of 75 sample of *Klebsiella*, 6% showed oxa positive. This study shows presence of various mechanisms responsible for carbapenem.

Keywords: Carbapenem, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Discovery Of Potential Drugs For Neurodegenerative Prion Diseases Using In Silico Approaches

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Abstract:

Prion diseases are fatal neurodegenerative diseases caused by conversion of a normal, cell-surface glycoprotein (PrPC) into a misfolded pathogenic form (PrPSc), which cause wide array of degenerative neurological disorders including human Creutzfeldt-Jakob disease (CJD), Bovine Spongiform Encephalopathy (mad cow), scrapie in sheep, goat, hamsters and mice, and chronic wasting diseases in deer. The possible strategy to inhibit PrPSc formation is to stabilize the native conformation of PrPC and interfering the interactions between PrPC and PrPSc. However, no promising drugs have been identified to cure prion diseases. Here, for the identification of potential anti-prion compounds we have integrated traditional ethnobotanical knowledge with modern in silico drug designing approach. Initially, we prepared in-house databases of 4008 molecules including bioactives from those plants with CNS stimulant activity and plants which are traditionally used to treat different diseases related to CNS, followed by docking in normal PrPC hotspots. Fifteen compounds with highest binding affinity were selected as lead compounds or potential anti-prion compounds and their pharmacokinetics profile (ADME/Tox) including Blood Brain Barrier analysis were predicted in silico. Some compounds which showed high binding affinity are bioactives of plants, which are traditionally used to treat different neurodegenerative diseases. These compounds might easily penetrate blood brain barrier and are capable of acting as pharmacological chaperones to stabilize native PrPC as well as may interfere the pathogenic conversion. Interestingly, these compounds might possibly be relevant for several neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease. Thus, for validation of these identified compounds, in vitro SPR, NMR, bioassays in ex vivo cell line cultures and in vivo mouse models are recommended.

Keywords: Anti-prion, Neuro Degeneration, Bioactives, Pharmacokinetics, Ethno Botanical

FIMM Drug Sensitivity And Resistance Testing Platform

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Abstract:

Cancer therapy is increasingly moving towards individualized care and therapy, but there are still big gaps between what is known and described on the molecular level about cancers and what is applied in the clinic. In an attempt to bridge the knowledge gap we at the Institute for Molecular Medicine Finland (FIMM) have set up an Individualized Systems Medicine program that integrates clinical, molecular profiling and functional information about individual patient's cancer. Central to this program is the Drug Sensitivity and Resistance Testing (DSRT) when we profile the responses of primary cancer cells to a comprehensive clinical oncology and signal transduction drug collection of 528 compounds.

Keywords: Drug Sensitivity and Resistance Testing (DSRT), FIMM High Throughput Biomedicine (HTB) unit, Individualized Systems Medicine

Detection Of Mutation At 1138th In Fgfr3 Gene In Transmembrane Region Of Achondroplasia Cases Of Nepal

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Abstract:

Achondroplasia (ACH) is the most common form of skeletal dysplasia of genetic origin in humans which is characterized by disproportionate rhizomelic dwarfism. Heterozygous mutation in the transmembrane domain of the FGFR3 gene (4p16.3) occurs as a de novo mutation in most of the cases. However, homozygosity is not well-suited to life (lethal in neonatal cases or die within months or years of birth). G to A ($\geq 95\%$) and G to C at nucleotide 1138 (G380R) is the most common point mutation in human genome.

DNA was isolated from 14 Achondroplasia patients from members of HochaPudka Association of Nepal. PCR was performed for the region incorporating the hotspot region viz. 1138th nucleotide. PCR amplicon of size 164 bps was obtained from all the samples which were then sent for sequencing. Based on their phenotype, the patients were classified as true Achondroplasia, Psuedoachondroplasia or Hypoachondroplasia. Sequence analysis showed the presence of mutation (G to A transition) in 6 of the 14 samples while the remaining samples did not show any mutation at the nucleotide. Of the 6 samples, 5 (35.71%) were designated to be true Achondroplasia while one sample was that of Pseudoachondroplasia. Information regarding this type of mutation in ACH to the high risk family will help to establish genetic test for diagnosis even during prenatal period such that the lives both of mother and child can be saved. It also helps in adding new evidence to the pool of the knowledge of mutation in Achondroplasia patients of Nepal.

Keywords: Achondroplasia, G1138A or C, G380R, Point Mutation, Fibroblast Growth Factor Receptor 3 (FGFR3)

Production Optimization Of Cellulase From Geobacillus Species Strain Kp43 Isolated From Hotspring Water

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Abstract:

Cellulases (EC 3.2.1.4) are complex hydrolases mainly comprising of exoglucanase, endoglucanase and β -glucosidase that act synergistically to breakdown cellulosic substrates. This study aims to produce cellulase from a thermophilic bacterium at optimum conditions of temperature, incubation period and pH while the culture is provided with most suitable C-source and N-source at optimum levels required for growth and enzyme production. A thermophilic cellulolytic bacterium was isolated from hot-spring water and the isolate was identified morphologically as well as by molecular techniques. Optimization of cellulase production was done by monitoring the cellulase production on different temperature, pH and incubation time and by varying media constituents like C- and N-sources. During each optimization, the previous optimum parameter obtained was used on the subsequent optimization of next parameter. The organism was found to be gram positive, thin, motile rod with terminal endospores and growing optimally between 55°C to 65°C and pH 5.5 to 6.5, which on phylogenetic analysis based on partial 16S rDNA gene sequence comparison showed high levels of similarity with *Geobacillus thermoleovorans*. Optimum production of cellulase was observed on incubation at 55°C for 18 hours in a medium containing Carboxymethyl cellulose (CMC) and yeast extract as C- and N-sources. Since, bacterial enzymes are expressed at relatively low levels, the optimization of different parameters is therefore important to augment the level of enzyme production.

Keywords: 16s rDNA, Cellulases, Hot Spring, Optimization, Thermophile

Serotyping Of Dengue Virus And Estimation Of T-Cells In Circulating Peripheral Blood Of Dengue Patients

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Abstract:

Dengue is a mosquito borne viral disease mostly transmitted through bites of infected *Aedes aegypti*. Epidemiological studies suggest that dengue has been rapidly spreading in tropical and sub-tropical countries imposing a significant health, economic and social burden. The geographical expansion of four dengue virus serotypes (DENV1-4) signifies the need for serotyping of dengue virus for proper surveillance and epidemiological studies. Dengue virus serotypes share 65% similarities in their genome but genetic variation can be observed even within single serotype. The understanding of changing pattern of dengue virus serotypes is important. Cellular immunity plays a vital role during dengue infection. CD8 T cells have been responsible for both protection and cytokine-mediated immunopathology in dengue patients.

Though various epidemiological studies regarding dengue has been carried out but the study about immunological response in dengue patients is minimal in Nepal. In this study, serotyping of dengue virus was performed and the change in pattern of serotype was observed. We used selective clinical samples available from two different hospitals of Nepal in 2016 and 2017 A.D. RNA was extracted from serum samples and Real-Time PCR was performed. The serum samples were collected from confirmed dengue cases. The flow cytometer analysis of dengue positive samples from 2017 was carried out for the study of immunological response in dengue patients in BD FACS Calibur. The panels were set with CD3 FITC, CD4 APC, and CD8 APC for the study of T cell subsets. The percentage of T-cells in dengue patient was estimated.

Thus, the change in pattern of dengue serotype was observed between two different years. DENV1 was found prevalent in 2016 whereas DENV2 may have prevailed in 2017. The percentage of CD3 count for six dengue positive samples were 44.6, 48, 59.3, 44, 44.5, 68.4 and that of CD4 were 35.9, 18.9, 25.1, 21.8, 25.2, 0.26 while that of CD8 were 15.4, 21.5, 27.3, 10.7, 33.5 respectively. Hence, Real-Time PCR is rapid and effective method for serotyping of dengue virus. There was a pattern change observed in dengue virus serotype in the samples of two consecutive years. The lower than normal percentage of CD3, CD4 and CD8 might be due to dengue infection.

Keywords: Dengue, Serotyping, DENV, Real time PCR, T cells

Isolation And Characterization Of Phosphate Solubilizing Bacteria Isolated From The Rhizosphere Of Coffee Plant And Their Effect On Coffee Seedlings

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Abstract:

Phosphorus (P) is one of the vital macronutrient required for the growth and development of plants. The low bioavailability of P in soil is major factor that limits growth and development of plant. A large number of microorganisms present in the rhizosphere are known to solubilize and make available the insoluble phosphorus in the available form to the plants. Phosphate solubilizing bacteria (PSB) isolated from rhizosphere of coffee plant may play an important role in improving phosphate availability for the plants. The objective of this study was to isolate and characterize PSB from coffee plant rhizosphere and their effect on growth and development of Coffee arabica seedlings. This research was carried out by taking soil samples from 6 months old coffee seedling. PSB were selected based on the quantitative ability to solubilize phosphate by measuring the clear zone surrounding the colonies in Pikovskaya's agar. In total, 26 PSB were isolated. Best 8 isolates (PSB 1, PSB 2, PSB 8, PSB 10, PSB 11, PSB 14, PSB 19, and PSB 20) were selected and their phenotypic and molecular characteristics (16S rDNA sequence analysis) were carried out. Best four isolates were selected for germination assay, seedling growth promotion assay and greenhouse trial. The effect of inoculation of PSB on germination of coffee seeds in-vitro was carried out and all isolates (PSB 1, PSB 10, PSB 11, and PSB 20) enhanced germination percentage (50-56%). In seedling growth promotion assay, PSB 11 and PSB 20 isolates enhanced shoot length, root length, shoot fresh weight and root fresh weight. In the green house trail experiment, PSB 1 and PSB 10 enhanced shoot length and root length of the coffee seedling.

Keywords: Coffee, Phosphate Solubilization, Phosphate Solubilizing Bacteria, Pikovskaya's Media, Rhizosphere

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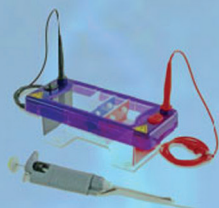
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