1ˢᵗ International Conference on

Recent Trends in Bioengineering
(ICRTB–2018)

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1st International Conference on

Recent Trends in Bioengineering (ICRTB–2018)

Editors
Prof. Vinayak Ghaisas
Dr. Renu Vyas

MIT SCHOOL OF BIOENGINEERING SCIENCES AND RESEARCH
MIT-ADT University, Pune

DISCLAIMER

Message

Dr. Vishwanath Karad

It gives me immense pleasure to learn that the ***First International Conference on Recent Trends in Bioengineering (IRCTB–2018)*** is being organized by MIT School of Bioengineering Sciences and Research (MITBIO) a constituent unit of MIT ADT University. Bioengineering is an upcoming field in India and has tremendous potential in industry as well as in research. It has always been the motto of MAEER MIT to impart excellent education and promote skill development among youngsters. I hope this conference will ignite young minds and encourage them towards research for benefit of the society.

I wish the organizers tremendous success and good wishes to all participants.

Prof. (Dr.) Vishwanath D. Karad
Founder MIT Group of Institutions
Founder & President
MIT ADT University

Message

Dr. Suresh Ghaisas

Bioengineering is an upcoming discipline which has tremendous potential in solving myriad problems in health care, environment, agriculture sectors in our country. Being a medical professional, I am eager to witness its growth in our country and wish that the results from research in this domain reach and benefit large sections of the society. MIT School of Bioengineering Sciences and Research is organizing an international conference on recent trends in Bioengineering and I strongly feel such events provide platforms that are required to help network our young generation with experts in the field and motivate them to pursue their studies and research with more vigour. My blessings and good wishes for the growth of the Bioengineering institute in general and this event in particular.

Dr. Suresh Ghaisas
President & Trustee
MIT MAEER

Message
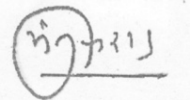
Dr. Mangesh Karad

Dear Delegates

My heartfelt welcome to all of you for the *International Conference on Recent Trends in Bioengineering (ICRTB–2018)*. I am sure you will benefit professionally form the technical conference and also enjoy our beautiful campus and local hospitality.

I congratulate the Bioengineering team for this initiative and wish the event supreme success.

Dr. Mangesh Karad
Executive President
MIT ADT University

Message

Dr. Sunil Rai

It gladdens my heart to know that our university is hosting an international conference on Bioengineering a one of its kind in the country. This conference is unique in that it is bringing together eminent professional from research, academia and industry from diverse disciplines that constitute Bioengineering. I deeply appreciate the organizational efforts of MIT School of Bioengineering Sciences and Research for creating this event. I extend my full support and best wishes to organizers and participants for this endeavour.

Dr. Sunil Rai
Vice Chancellor
MIT ADT University

Message

Prof. Vinayak Ghaisas

It gives me immense pleasure to have our Bioengineering institute host the *International Conference on Recent Trends in Bioengineering, 2018*. This is certainly another step towards fulfilling our vision of advancing human health & wellbeing of our society. The primary purpose of the conference is to exchange ideas and improve upon the collective knowledge and wisdom of the society. Our institute course work is carefully designed ground up to deliver on our vision. We are imparting knowledge to our students to make them industry ready; We are inculcating research oriented thinking, problem solving skills and also making them "financially-literate" and provide them with opportunities & ecosystem to incubate new ideas and launch new enterprises. We are confident that with this approach, India as a country will become hub of Bioengineering entrepreneurs.

I am sure all the attendees of the conference will benefit tremendously by exchange of ideas and networking with other scientists.

Welcome and Best Wishes.

Prof. Vinayak Ghaisas
Founder Director & Convenor
MIT School of Bioengineering Sciences & Research

Message

Dr. Renu Vyas

The present era belong to biology as almost all major innovations, technologies, research being reported either have applications in biology or draw inspiration from it. The theme of this conference is to highlight the current frontier areas of research Bioengineering mainly with respect to the three major sub-disciples viz. biotechnology, bioinformatics and biomedical engineering. Bringing together and linking such major disciplines under a single umbrella for this international conference is a humongous task but we have strived hard to make it happen.

I hope the conference will enthuse the young faculty, research scholars, students for ideation and application of biology in design and development of new safe and efficacious medicines, healthcare assistive devices for elderly and differently abled, new biomarkers, algorithms etc. to enrich the field further. I am sure all the organizers, eminent speakers, guests and delegates will participate enthusiastically in the conference and the memories of the event will be cherished forever.

Thanks everyone and warm welcome,

Dr. Renu Vyas
Head of School & Convenor
MIT School of Bioengineering Sciences & Research

[x]

Summary of Plenary Talk

AI for applications in neurology and psychiatry

Prof. Justin Dauwels
NTU, Singapore Deputy Director,
ST Engineering–NTU corporate lab

Many tasks in medicine still involve substantial manual work. In many cases there is strong potential for intelligent automation by A.I., leading possibly to a reduction in costs and man-hours, while increasing the quality of clinical service. In this talk, we will consider applications of A.I. in the domain of neurology and psychiatry.

Specifically, we will give an overview of our research towards automated interpretation of EEG signals (for diagnosis of epilepsy), and automated behavioural analysis for assessing the negative symptoms of mentally ill patients.

Summary of Talk

Affordable Medical Devices: Idea to Impact

Prof. B. Ravi
Institute Chair Professor, Mechanical Engineering Head,
Biomedical Engineering & Technology Incubation Centre
Indian Institute of Technology Bombay, Mumbai

There is an immense potential to leverage emerging technologies for solving pressing healthcare needs of humanity. This requires multi-disciplinary collaborations: unmet need identification by doctors, focused research by scientists, product development by engineers and commercialization by entrepreneurs. Together, they need to traverse the 'Valleys of Death' from idea to prototype, prototype to product, and product to market. In this short talk, we share a few success stories and best practices from BETiC network (www.betic.org). During the last three years, their team members curated 400 unmet clinical needs, evolved proof-of-concepts of 100 different devices, developed and filed patents for 30 products, and licensed 10 to startups, Indian industry, NGOs or hospitals. These include surgical instruments (laparoscopic cauterizer, nasal osteotomy forceps, aortic valve leaflet template), diagnostic devices (smart stethoscope, Clubfoot brace monitor, diabetic foot screener, glaucoma screener, biopsy gun), and prostheses & rehab aids (nasal implant, prosthetic leg, orthopedic surgery jigs and bone scaffolds). Some of the products have won prestigious awards and funding support from various agencies. The innovators, who left lucrative jobs elsewhere, are becoming 'job creators' (instead of 'job seekers'). They are also inspiring and mentoring innovators in other institutes, creating a sustainable eco-system for local design and manufacture of affordable medical devices.

Summary of Talk

NISCAIR activities

Dr. Sanjay Sengupta
CSIR-NISCAIR

The National Institute of Science Communication & Information Resources (NISCAIR), a constituent of CSIR, has been involved in dissemination of information for S&T community through its various scientific journals. NISCAIR has been making a major contribution to Indian Science by publishing as many as 18 journals. These Journals are of international repute and provide communication links among members of the scientific community engaged in research in India and abroad. NISCAIR publishes 18 scholarly Journals viz: JSIR (Monthly), IJBB (Bimonthly), IJEB (Monthly) BT (Quarterly), IJMS (monthly), IJTK (Quarterly), JIPR (Bimonthly), NPR (Bimonthly) IJPAP (Monthly), IJFTR (Quarterly), IJC-A (Monthly), IJCB (Monthly), IJCT (Bimonthly) IJEMS (Bimonthly), IJRSP (Bimonthly), MAPA (Bimonthly), ISA (Fortnightly) and ALIS (Quarterly).

- Writing Scientific Papers

- About JSIR

- Science Communication.

Summary of Talk

Robotic Surgery

Dr. Shailesh Puntambekar
Medical Director, Galaxy Hospitals

Everyday there is a development, a progress made in the field of science and technology. When it comes to healthcare the greatest leap in technology occurred in minimally invasive surgery. The normal surgery was performed by one chief surgeon and observed by a few handful learning doctors, today all the learning is just a click away. Major surgeries today are performed with the smallest of incisions on the body. Laparoscopy is the greatest leap in the field of surgery. With further advancements in technology involving better sharper and more precise instruments, it aided the surgeon for better or superior dissection of tissues while conducting the surgery. However with surgery lasting for hours together, this took a toll on surgeons and this became a necessity to make sure all surgeries precise, safe and not affecting the operating surgeon. Like every necessity giving birth to invention, this gave birth the robotic surgery systems.

Robotic surgery is surgery conducted on the same principles of laparoscopic surgery with the precision and safety features of a robot. the surgeon conducting the operation is still human but the finer aspects of human error are reduced with the help of robotic surgery. Robotic surgery ensures finer, precise and accurate movement without compromising the health of the patient at any given period of time. The next leap in Robotic surgery is telesurgery. With two robots sycnhronised in two different places, tele surgery helps conduct surgeries all over the world without the doctor needing to physically transport to various places. These advances in technology have shaped human lives in many ways. and with the proceeding time advancements in technology don't seem to cease or decelerate their pace.

Summary of Talk

Agricultural Biotechnology: Potentials and Applications

Dr. Narendra Kadoo
Principal Scientist, Biochemical Sciences Division,
CSIR-National Chemical Laboratory, Pune

Agricultural biotechnology includes molecular biology tools and techniques like genetic engineering, molecular markers, molecular diagnostics, tissue culture, etc. to modify crop plants, animals and microbes for increased productivity, quality and resistance to biotic and abiotic stresses. Humans have been using biotechnology unknowingly for thousands of years and developed fermentation technology to produce and preserve foods like breads, cheese, yogurt, alcohols, etc. They also domesticated plants and animals, and performed selective breeding for increased productivity. In recent times, several transgenic plants, animals, fishes and microbes have been developed through genetic engineering, which have the novel traits. The talk will focus on some of these technologies to develop crop plants that are resistant to biotic and abiotic stresses.

Summary of Talk

Design, Discovery and Development

Dr. Bhalchandra Vaidya
Manager (R&D), Gennova Biopharma Ltd., Pune

Vaccination is one of the most effective method for prevention of several infectious diseases. A vaccine provides active acquired immunity against a particular disease. Vaccine adjuvants are molecules or macromolecular complexes that boost the potency and longevity of specific immune responses to antigens. The addition of adjuvants to vaccines enhances, sustains and modulates the immune responses which ultimately improves the vaccine efficacy and reduces the amount of antigen per dose and also immunization frequency. This presentation will provide an overview of current status and future directions of adjuvant design and development.

Adjuvants have been used to improve vaccine efficacy from the early 1920s. Despite many decades of research, only limited number of adjuvants are used in clinically approved vaccines for human use. Adjuvants based on insoluble aluminiumsalts, oil in water emulsions and liposomes have been used inhuman vaccines since many years and have seen significant success as components of licensed products. Recent commercial interests in adjuvant design and development is driven by three major factors. The first one is associated with recent mechanistic understanding of innate immunity and immunological memory. These theoretical insights provides a foundation for rational adjuvant discovery. Secondly, current focus on the development of safer sub-unit vaccines over conventional live-attenuated or inactivated vaccines must be taken in to account. Unlike traditional vaccines, sub-unit vaccines often lack sufficient immunogenicity and need to be administer with adjuvants. Finally, several efforts in developing novel vaccines for challenging diseases like malaria, tuberculosis, HIV have emphasized the limitations of currently available adjuvants and highlighted the need for novel adjuvants that are capable of boosting the specific types of T-cell immune responses (specifically CD4 and CD8 T-cell responses). Safety considerations, improving adjuvant's capability to trigger multi-faceted immune responses, analytical characterization of adjuvants, formulation optimization, cost-competitiveness and most importantly meeting regulatory compliance are major challenges in adjuvant development.

Summary of Talk

Dr. Virendra Ghaisas

Executive Medical Director, Professor–Department of ENT,
M.I.M.E.R. Medical College, Talegaon Dabhade

Dr. Virendra Ghaisas will speak on Bioengineering scope in context of medical field applications. He has extensive experience in rhinoplasty. He has chaired many Otorhinolaryngology and head neck surgery sessions in national and international conferences as well as at various AOI chapter meetings. He is the recipient of Dr. A. R. Bhide Memorial Award twice at MENTCON.

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Program Schedule

INAUGURAL SESSION: (9–10.00 A.M.)

8.15–9.00 am	Registration, kit distribution and hosting the guests and delegates
9.00–9.05 a.m.	Welcoming the audience by the anchor, announcement of senior guests list
9.05–9.10 a.m	World peace prayer
9.10–9.15 a.m	Ganesh Vandana (a Bharatnatyam dance recital)
9.15–9.20 a.m	Saraswati Pooja and lighting of the lamp by dignitaries on dais
9.20–9.25 a.m	Introductory address by Prof Vinayak Ghaisas Convener, Director and Founder, MIT *School of Bioengineering Sciences & Research*
9.25–9.30 a.m	Conference theme talk by Dr. Renu Vyas, Convener and Head of School, *MIT Bioengineering Sciences & Research*
9.30–9.35 a.m	Welcome address by Dr. Sunil Rai, *Vice Chancellor, MIT ADT University*
9.35–9.40 a.m	Speech by Dr. Mangesh Karad, executive president, *MIT ADT University*
9.40–9.45 a.m.	Talk by Dr. Virendra Ghaisas on changing scenario of medicine and relevance of Bioengineering
9.45–9.50 a.m	Inspirational talk by Hon Vishwanath Karad sir, founder MIT group of institutes.
9.50–9.55 a.m.	Introduction and Felicitation of all invited speakers and industry guests by Hon. Vishwanath Karad and release of abstract book of conference and CD.
9.55–10.00 a.m	Vote of thanks
10 a.m.	Tea Break and group photo with all delegates outside the RK auditorium

TECHNICAL SESSION I: (10. 15 A.M.–12.30 P.M.)

Session Chair: **Dr. Ravi, IIT Bombay**

10.15–10.40 a.m	Plenary talk by Dr. Justin Dauwels, Professor NTU, Singapore on Machine learning and data science
10.40–11 a.m.	Invited talk by Dr. ShaileshPuntambekar, Director, Galaxy Hospitals on robotic surgery
11. 15–11.30 a.m.	Perspective talk by Dr. GurmukhAdvani, MD Getinge group on Biomedical device industry in India
11.30–11.45 a.m.	Technical talk on biomedical implants by Mr. Netaji Khot , Orchid Diagnostics
11.45–12 p.m.	Dr. Ravi, BETiC head, IIT Bombay on biomedical devices and point of care diagnostics
12.–12.30 p.m.	Panel Discussion, QA session/ interaction with audience

BETiC LAB INAUGURATION (12.30–1.45 PM)

12. 30–12. 45 p.m.	Inauguration of BETiC Lab by Dr Ravi, IIT Mumbai and his team in presence of all dignitaries in first floor MITBIO building
12. 45–1. 45 p.m.	Lunch, poster session and industry stalls exhibition

TECHNICAL SESSION II: (1.45 P.M.–3.00 P.M.)

Session Chair: **Dr. Narendra Kadoo, Scientist NCL**

1.45–2.00 pm	Dr. Ashok Giri, Chair, Biochemical Division, NCL Pune , emerging trends in Biotechnology
2.00–2.15 pm	Dr. Narendra Kadoo, Biotechnology in agriculture
2.15 to 2.30 pm	Dr. Bhalchandra Vaidya, DGM Gennova Biotech on vaccine design
2.30–2.45 pm	Panel discussion and interaction with audience
2. 45–3.00 pm	Dr. Sanjay Sengupta, CSIR-NISCAIR on NISCAIR activities and guidance to students regarding publishing research work
3. 00–3.15 pm	Tea Break, poster evaluation and industry stalls visit

TECHNICAL SESSION III: (3.15 P.M.–4.45 P.M.)

Oral Presentations by: **Research Scholars/ Faculty**

3.15–3. 45 pm	Oral presentations in Biotechnology, Experts: Dr. Ashok and Dr. Kadoo
3.45 to 4.15 pm	Oral presentations in Bioinformatics , Experts: Dr. Renu Vyas and Dr. Justin Dauwels
4.15–4.45 pm	Oral presentations in Biomedical engineering; Experts: Dr. Ravi and Dr. Netaji Khot

VALEDICTORY FUNCTION (4.45–5.00 PM)

4.45–4.50 pm	Announcement of Bioengineering Society Charter by Dr. Sunil Rai
4.50–05 pm	Prize distribution for best oral presentation first and second prize, best poster presentation first and second prize at the hands of Director, Vinayak Ghaisas
05 pm	Tea and snacks

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BI

BIOINFORMATICS (ORAL PRESENTATION)

Subtractive Genomics Approach Used in In-Silico Drug Target Identification for the ⋆Streptococcus Pyogens ⋆⋆Serotype M1

Shilpa Siragannavar

Akkamahadevi Womens University, Vijayapur, Karnataka

ABSTRACT—The present work was carried out to find out the drug target in streptococcus species. Streptococcus pyogenesis a gram positive organism that causes many human infections It causes mild superficial skin infection like pharyngitis and localized skin infection impetigo and severe life threatening infections like necrotizing fasciitis. Here in this study Subtractive genomics is used to identify the new potential drug target for pathogenic organism. Subtractive genomics is a technique of subtraction of genes between host and pathogen which gives the set of essential genes which are important for pathogen survival but absent in host. Various Bioinformatics tools were used to subtract the host and pathogen genes. The objective of the work was to identify and locate the essential proteins of S.pyogenes that are unique i.e. absent in host and performing normal function within the host and to shortlist them in vaccine development point of view. Further analyzing these essential and non-human homolog genes, it was found that a protein that is possibly located on the membrane of the pathogen could be considered as potential drug target for the pathogen.

INTRODUCTION

Streptococcus pyogens is a gram positive bacterium that causes many human infections. It causes mild superficial skin infection like pharyngitis and localized skin infection impetigo and severe life threatening infections like necrotizing fasciitis. Until the advent of the antibiotic era, Group A *Streptococcus* was a major cause of death in industrialized countries as a result of sepsis, rheumatic heart disease and fatal epidemics of scarlet fever [1]. *Streptococcuspyogenes*, also known as group A streptococcus (GAS), is most commonly associated with mild, self-resolving infections of the skin and oropharynx. However, dissemination of the bacteria to normally sterile sites within the body can lead to a variety of invasive conditions that are associated with high morbidity and mortality [2]. Proteomic approaches are also used to identify immune reactive proteins for vaccine development and to identify proteins that may induce autoimmunity [3]. Overall, *S. pyogenes* causes in excess of half a million deaths each year worldwide, which is similar to that of *hepatitis B or H. influenzae* [4]. There is no licensed vaccine to prevent disease and while the pathogen remains sensitive to β-lactam antibiotics, it has relatively recently acquired resistance to macrolide antibiotics [5, 6]. Thus, the current burden of *S. pyogenes* on human health is significant and a better

understanding of the *S. pyogenes* proteome–the molecules largely responsible for mediating host–pathogen interactions–is essential for developing new treatment strategies and vaccines [7]. A modern approach called "Subtractive genomics" is currently widely engaged to identify novel and specific drug targets in pathogenic organism, as a step towards identifying novel and potential drugs [8]. Completion of Human Genome Project is one of the major revolutions in the field of drug discovery against human pathogen. At present time genomic approach is in tradition. Identification of novel therapeutic targets is one of the major tasks in order to design a novel drug [9]. Several antibiotics have many side effects and developed resistant against Streptococcus species. Computational subtractive genomics approaches, based on the strategy that an essential survival protein non-homologous to any human host protein is a candidate drug target for a given parasite, have been successfully used to identify putative drug targets in *S.pyogens*, *Pseudomonas aeruginosa, H. pylori, B. pseudomallei, and A. hydrophila*[10]. Metabolic pathway analysis of the essential proteins of *Mycoplasma pneumoniae*was done by KAAS server at KEGG (http://www.genome.jp/tools/kaas/) for the identification of potential targets. KAAS (KEGG Automatic Annotation Server) provides functional annotation of genesby BLAST comparisons against the manually curated KEGG GENES database. The result contains KO (KEGG Orthology) assignments and automatically generated KEGG pathways [11]. Computational prediction of bacterial protein subcellular localization provides a quick and inexpensive means for gaining insight into protein function, verifying experimental results, annotating newly sequenced bacterial genomes, and detecting potential cell surface/secreted drug targets. Subcellular location is considered as one main criterion for vaccine target prediction [12]. Molecular docking has played key role in the identification of efficient binding of receptor and ligand [13].

METHODOLOGY

RETRIEVAL OF PROTEOMES OF HOST

The complete proteome of pathogen 1,690, "*Streptococcus pyogenes* serotype M1 [301447]" AND proteome: UP000000750 was retrieved from the Uni Prot. The flow chart for different steps performed in the current study is given in Fig. 1.
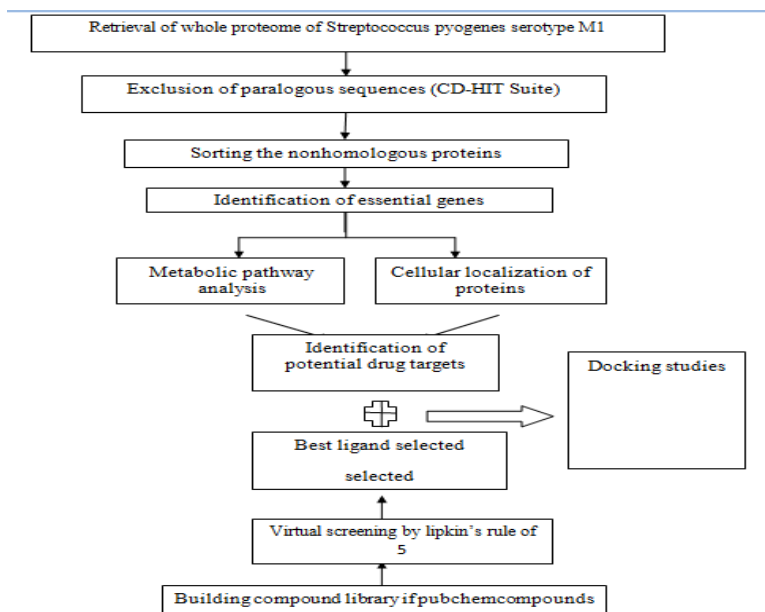
Fig. 1: Schematic Diagram of the Flow Chart for Drug Target Identification and Ligand Binding, Docking Studies of Target and Ligands

Fig. 2: Retrieval of All the 1690 Protein Sequences of the Microbe, Streptococcus Pyogenessero Type M1

IDENTIFICATION OF DUPLICATE PROTEIN INPATHOGEN

The whole proteome of *Streptococcus pyogenes*serotype M1 was subject to selective removal by CD-HIT Suite (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/). with

the sequence identity cut-off at 60% [14] to remove all aralogous proteins or duplicate proteins within the pathogen, *Streptococcus pyogenes*. The paralogus were excluded and the remaining sets of protein dataset was used for further analysis and referred to as set no. 1 proteome.
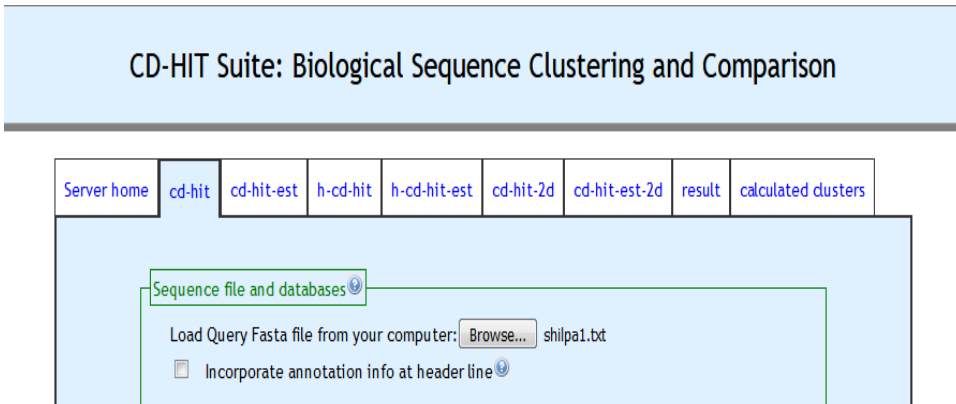
Fig. 3: Selective Removal of the Duplicate Proteins by CD-HIT Suite

IDENTIFICATION OF NON-HUMAN HOMOLOGUES PROTEINS BY PERFORMING BlastP

The nonparalogus proteins were subjected to NCBI BlastP against Homo sapiens protein sequences using threshold expectation value (E–value) $<=10^{-5}$ as parameter to find out the non-human homologues proteins of *Streptococcus pyogenes*. The human homologs were excluded and the list of non-homologs was compiled. This further filtered data set proteome was referred to as dataset no. 2, which do not have any homologous proteins to those of *Homo sapiens*.
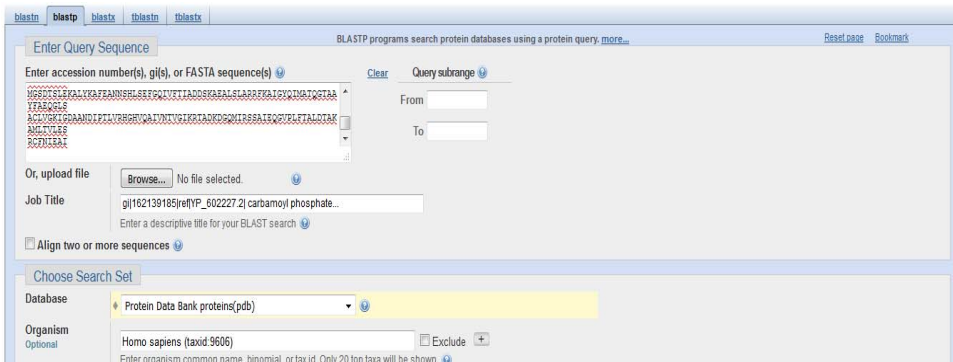
Fig. 4: Running BlastP of the Microbe Proteins with PDB Database Against Homo Sapiens Protein Sequences

[6]

IDENTIFICATION OF ESSENTIAL PROTEINS FROM NON-HUMAN HOMOLOGUES PROTEINS THROUGH COMPARISION WITH DEG

The selected nonhuman homologues proteins (datset no. 2) are now subjected to similarity search using standard NCBI against the Database of Essential Genes (DEG) in which genes essential for the survival of *Streptococcus pyogens* were selected as the reference. A random expectation value (E-value) cut-off of 10-100 and a minimum bit-score cut-off of 100 were used to screen out proteins to represent essential proteins [15].

Thus, the resulting essential protein sequences (dataset no.3 proteome) obtained were non-homologous to Homo sapiens proteome and represent a way to subtract the host proteome from further analysis. The critical genes required for the survival of the pathogen are identified using Database of Essential Genes (DEG).

Fig. 5: Identification of the Critical Genes Required for the Survival of the Pathogen are Identified using Database of Essential Genes

ANALYSES OF METABOLIC PATHWAY(S)

The essential proteins (dataset no. 3) of *Streptococcus pyogenes*as identified in the previous steps are subject to metabolic pathway analysis using KAAS at KEGG for the identification of potential targets. KAAS provides functional annotation of genes by BLAST comparisons against the manually curated KEGG GENES database. The genes and their products which can be used as a potential drug targets can be identified by analyzing these genes with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database [11, 16].
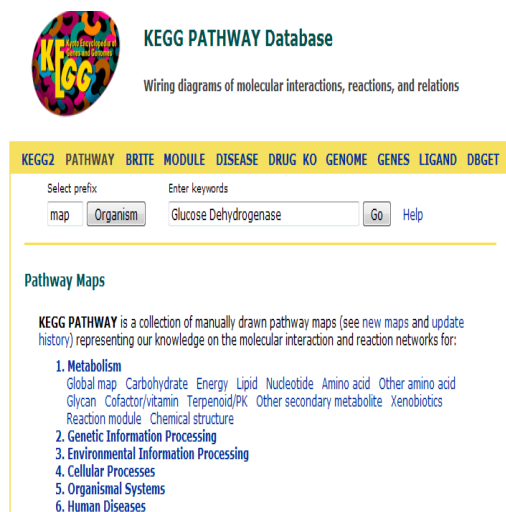
Fig. 6: The Essential Genes were Subject to Metabolic Pathway Analysis using KAAS at KEGG for the Identification of Potential Targets

PREDICTION OF SUB CELLULAR LOCATION

Protein localization prediction of essential proteins is an important step to predict the protein function and it can be very useful for the identification of targets. Sub-cellular localization analysis of the essential protein sequences was done by SOSUI SERVER [17], to identify the membrane surface proteins which can be feasible for effective target identification.

BUILDING A COMPOUND LIBRARY AND FINDING A GOOD LEAD BY SCREENING LIBRARY OF COMPOUNDS, VIRTUAL SCREENING TO DETERMINE BEST LEADS (LIGAND) TO TARGET

Virtual screening is done to determine the best lead which binds at appropriate sites with target to enhance the rate of reaction. Virtual screening is a computational technique used in drug discovery research. By using computers, it deals with the

quick search of large libraries of chemical structures in order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme. The aim of virtual screening is to identify molecules of novel chemical structure that bind to the macromolecular target of interest [18].

BUILDING OF COMPOUND LIBRARY

A compound library was generated by collection of around 100 compounds with the antibacterial activity. The 3D structure which were downloaded from Pubchem, and Chemspider as SDF or Mol files. The target obtained for *Streptococcus pyogenes*is based on annotations as well as minimum energy conformation of compounds which was derived using Marvin Sketch. Lipinski's screening based on Lipinski's rule of 5 for the obtained scores like Molecular Weight. H-Bond Donor, H-Bond Acceptor, XLogP. The molecular weight of the compound selected should be less than 500g/mol, H-bond donor should be less than H-acceptor should be less than 10, &Xlog P should not be more than 5. Based on the Lipinski's rule of 5 the leads are screened [19]. These screened leads were used for Auto Docking. Using Auto DockVina all compounds were docked one by one with the target obtained.

MOLECULAR DOCKING ANALYSIS OF THE TARGET OBTAINED WITH THE SCREENED LIGANDS

Auto DockVina is a new open-source program for drug discovery, molecular docking and virtual screening, offering core capability, high performance and enhanced accuracy and ease of use. The target structure was obtained by homology modeling using SWISSMODEL server [20]. Auto DockVina used to select target molecule and add a grid box with particular dimensions by preparing conf.txt file, grid box was optimized to cover the whole area of the target. The energy of interaction of the ligands with the targets was assigned "grid point." Docking scores for each ligand were noted. And based on the existing drug docks scores, the best dock scores were selected.

TO STUDY ADMET PROPERTIES AND TOXICITY OF THE LIGANDS

The online tool pre ADME was used to study ADMET properties and toxicity of selected compounds. Results of all tools are attached in results section. The structures of the leads are included in the results section [21].

RESULTS AND DISCUSSION

The current work describes a subtractive genomics approach for identification of a suitable drug target among the essential proteins from the total proteome of

Streptococcus pyogens. The subtractive genomics approach has been reported as an innovative and powerful method for identifying unique sequences as potential therapeutic targets [22]. The subtractive genomic analysis is sorting of the essential proteins of a pathogen as identifying unique proteins that are absent in the host organism in order to facilitate drug designing by avoiding the toxicity of the host through cross-reactivity with *Homosapiens*proteome.

The *Streptococcus pyogenes*serotype M1 contains a total of 1690 proteins in its proteome. 1067 proteins were found to be duplicates or paralogs with 60% identity from the analysis with CD-HIT suite and were eliminated from the dataset as these were redundant as drug targets. The remaining 623 proteins set no. 1 proteome were analyzed using BlastP against a customized human protein database and were again excluded from the dataset no. 1 as these proteins may cause drug cross-reactivity and host cytotoxicity when used as drug targets during its treatment. The resulting set no.2 proteome containing 664 proteins were used for further analysis and subject to BlastP search in the database of essential genes (DEG) in order to determine the essential genes required for the survival of the pathogen. A total of 330 proteins were found to be essential, which means that these proteins are involved in metabolic pathways indispensable for the propagation of this pathogen and thus can be used as target for treatment options (Table 1).

Table 1: Subtractive Genomic and Metabolic Pathway Analysis Result for *Streptococcus Pyogenes*

Total Number of Proteins	1690
Duplicates (>60% identical) in CD-HIT	1067
human homologous	623
Non-human homologous proteins (E-value 10^{-5})	664
Essential protein in DEG (E-value 10^{-5})	330
Essential proteins involved in metabolic pathways	18
Proteins involved in unique pathways	5
Membrane associated drug targets finally obtained	1

METABOLIC PATHWAY ANALYSIS

The 330 proteins which are to be essential as analyzed through the DEG were passed through the KEGG-KASS server to analyze their metabolic pathway. It was found that 5 proteins were involved in metabolic pathways unique to the pathogen and thus, not found in humans.

The localization of the protein plays important role in determining the target, hence the targets suggested based on the SOSUI results, i.e location of the target and the best selected target was Peptidoglycan Hydrolase.

The 3D structure of the target was obtained by tertiary structure prediction method Homology modeling [23]. For homology modeling Swiss model was used the fasta

sequence of the Peptidoglycan Hydrolase was checked for homologous sequences using Blastp. Based on the identity template sequence was obtained and then the template sequence was used to get the structure of the target using alignment mode. The Qmean tells the stability of the model as shown in the Fig. 7. Validation of the model was done by RAMPAGE, a Ramachandran Plot Analysis, the evaluated residues expected percent was 98% which suggests that the obatained model is good for further analysis.
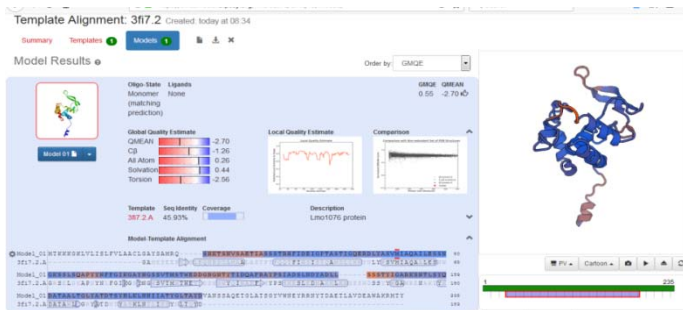
Fig. 7: Generation of the Target Model Using Template by Swiss Model
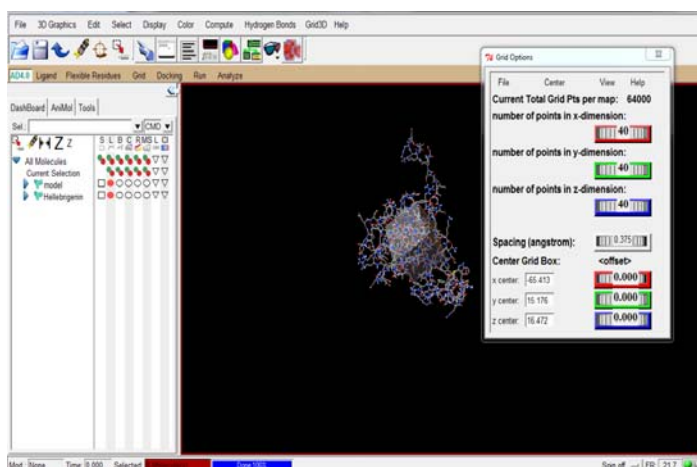
Fig. 8: Grid Box Setting for Target and Ligand Toprepare Protein and Place it in a Fixed Position for the Ligand to Bind

MOLECULAR DOCKING OF THE PROTEINS

The current study was further reinforced by performing comparative docking studies of the novel proteins with the ligands from ligand library. The molecular docking study was performed on protein target name Peptidoglycan Hydrolase and a set of 10 chemical compounds retrieved from pubchem database using both Auto

dockvina and Patchdock [24]. For creation of gpf, grid parameter file and dpf, docking parameter file using Autodock, the polar hydrogen atoms, Kollman charges parameters were set. For ligand the preparation before docking were done like detecting and choosing tortiontree. Grid setting plays an important role in docking because here one can decide the XYZ coordinates of the amino acids in the target for ligand binding. Binding affinities from docking were compared between target protein and against the corresponding chemical compound are tabulated in the table 2. Top docking pose, consider the binding energy (lowest) E-total, and the affinity interactions with active site of the protein. Since the E-total of the compound Id cid_259577 is -0.01, i.e Hellebrigen In was the effective ligand obtained. The grid box was set with X-centre: -65.143, Y-centre: 15.173, and Z-centre: 16.472 as shown in the Fig. 8 below.
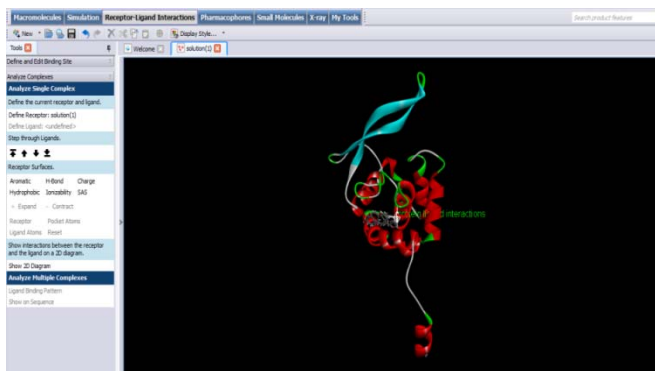
Fig. 9: Ligand Target Docking Using Patchdockto know the Binding Sites in the Target Protein Peptidoglycan Hydrolase With Ligand Hellebrigenin
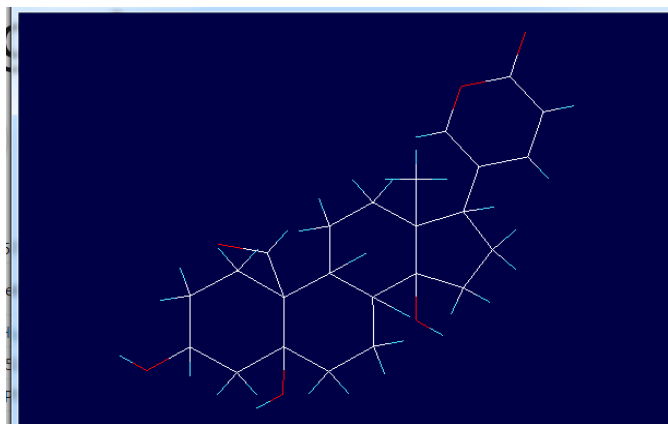
Fig. 10: Structure of Ligand Hellebrigenin, Pubchem Id cid_259577

**Table 2: The Affinity and Binding Energy Scores Obtained for
the Selected 10 Ligands for Docking with the Taget**

Compound Id	Affinity		E-Total
cid_259577	-10.3		-0.01
cid_5351344	-10.2		-0.04
cid_83843	-10.2		-345.28
cid_105111	-10.2		-221.65
cid_54678486	-9.0		-552.60
cid_4042	-8.3		-21.49
cid_8530	-7.9		-306.81
cid_9064	-7.2		-262.81
cid_382831	-6.9		-306.81
cid_9817550	-5.4	-471.78	

TARGET INFORMATION

Most bacteria have multiple peptidoglycan hydrolases capable of cleaving covalent bonds in peptidoglycan sacculi or its fragments. An overview of the different classes of peptidoglycan hydrolases and their cleavage sites is provided. The physiological functions of these enzymes include the regulation of cell wall growth, the turnover of peptidoglycan during growth, the separation of daughter cells during cell division and autolysis. Specialized hydrolases enlarge the pores in the peptidoglycan for the assembly of large trans-envelope complexes (pili, flagella, secretion systems), or they specifically cleave peptidoglycan during sporulation or spore germination. Moreover, peptidoglycan hydrolases are involved in lysis phenomena such as fratricide or developmental lysis occurring in bacterial populations [25].

CONCLUSION

With the advent of knowledge obtained from genome projects, a vast array of information regarding the proteomes and genomes of various prokaryotic, eukaryotic organisms as well as the human can be manipulated to accelerate the drug designing and drug discovery. Running BlastP against human allowes in determining the non human homologs sequences. The DEG database is more efficient than other methods for identification of essential genes which facilitates the exploratory identification of the most relevant drug targets in the pathogen. The present study has thus led to the identification of protein that can be targeted for effective drug design and vaccine development against *Streptococcus pyogenes*serotype M1. The further knowledge of pharmacogenomics can be used in the treatment of bacterial infections since the drugs developed will be more specific to the pathogen, and preferably less or not toxic to the human. The number of essential genes in the metabolic pathways of *Streptococcus pyogenes*serotype M1, identified in the present study, is relatively less i.e 18, these were further characterized to unique pathways to

find their role in the survival of the bacteria. Homology modeling of these targets help in identifying the best possible sites for ligand to be targeted for drug design by simulation modeling. Virtual screening against the target is useful in the discovery of novel therapeutic ligands against *Streptococcus pyogenes*sero type M1. In the current study outer membrane protein is considered as drug target because *Membrane-associated* proteins are excellent *targets* for diagnostic and therapeutic interventions. The drug obtained from the study does not show cross reactivity with human proteins. Thus Subtractive genomics helps in the identification of potential drug targets, by analyzing the results it can be said that subtractive genomics approach can be effectively applied to identify drug targets in pathogenic organisms for specific host organism.

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Designing and Development of Novel PARP-1 Inhibitor Involved in Ovarian Cancer

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ABSTRACT—*Poly (ADP-ribose) polymerase-1 (PARP-1) is an enzyme which has critical role in DNA replication repair and recombination the nuclear enzyme play an important role in the genomic repair process. PARP-1 is abundant and best characterized member of the PARP superfamily and has emerged as a promising molecular target for the treatment of ovarian cancer. Rucaparib is a potent drug for mammalian poly (ADP-ribose) polymerase (PARP) 1, 2 and 3 inhibitor with anticancer properties. The current study deals with in silico discovery of novel inhibitor against PARP-1 target. As the molecular level study of binding mechanism of Rucaparib with target protein were carried out by molecular docking studies with PARP-1 inhibitor and it defining a potential predictive biomarker for expanding the field of PARP-1 inhibitor therapy. As the new lead of the targeted drug were derived and design by virtual screening and analysis of their revealed hydrophobic contact as a major contributing factor. Also the further the studies deals with the profile proteomic patterns in the body fluid and provided a novel and highly sensitive diagnosis tool for the early detection of ovarian cancers. Numerous proteomics-based molecular biomarkers/panels have been identified and grasping great potential for diagnostic applications, need for further development and validation proteomics analysis of early stages cancers.*

KEYWORDS: *Rucaparib, Molecular Docking, Poly (ADP-Ribose) Polymerase-1 (PARP1), Ovarian Cancers*

INTRODUCTION

Epithelial ovarian cancer (EOC) is the prominent cause of death in women among all gynaecological malignancies, secretarial for about 5% of all the cancers and in that 4.2% of all cancer deaths in women worldwide are the most frequent type of ovarian cancer [1]. Ovarian cancer still characterizes the main cause of death in women with gynaecological cancers. The occurrence of ovarian cancer among gynaecologic malignancies is escalating; appropriately, most of patients are analysed at advanced stages with subsequently worse prognosis generally persistence is the poorest of all gynaecologic malignancies, with a five-year relative survival rate of 44% for all stages [7]. For an extended period of time, ovarian cancer has been well-defined as one single disorder. Nowadays, ovarian cancer is a heterogeneous disease which includes various biological manners from a clinical and molecular point of view. Epithelial ovarian cancer is categorized by four main histotype that show distinction resembling normal tissues of genital apparatus. Ovarian cancer seems to originate from the cells that line of the fallopian tube, endometriosis tumours from mucinous tumours end cervix then clear cell tumours from the vagina epithelium. Even from a molecular consideration the genetic profile of an each histotype is

analogous to that of the histological complements in normal cells [7]. On this basis, Kurman *et al*. has recently considered the function of the ovarian surface epithelium in tumorigenesis of epithelial ovarian cancer. The role of fallopian tube in the pathogenesis is serous ovarian carcinomas besides foci in endometriosis and clear cell ovarian cancers [8]. Epithelial ovarian cancer is collected of a heterogeneous group of tumours. The four most predominant subtypes are serous, endometriosis, clear cell, and mucinous carcinoma. Less common are provisional cell tumours, including transitional cell carcinoma and malignant Brenner tumour [2]. Encourage of amelioration in the recognition and cytotoxic therapies, only a self-effacing increase in the expectation rate beyond five years after early diagnosis of ovarian cancer has been proficient. There are numerous factors responsible for the high fatality rate, containing the insufficiency of any distinctive manifestations in the initial stages of an ovarian cancer; as the late diagnosis, which becomes a problem in designing an intervention; and the development of chemo resistance in cancer cells. Thus, it becomes imperative to improve screening approaches for EOC detection at early stage, as well as effective treatment for progressive stages of ovarian cancer patients [1]. The first line treatment including of platinum/taxable shows a decent response rate, but the occurrence of reappearance of the disease is common. In addition, the second line treatments are not effective in treating this disorder [3]. In improvement of avant-garde biomarkers for each subtype of ovarian cancer has become a demand for designing a better and more diligent cure approach for ovarian cancer [1]. Poly (ADP-ribose) polymerases (PARPs) inhibitors are a group of chemical compounds that are being developed for cancer treatment under the concept of synthetic lethality. PARPs are enzymes that transfer ADP-ribose moieties to a variability of protein substrates [4]. The physiological function of ADP-ribosylation is best characterized in the framework of genome permanency maintenance, in which ADP-ribose polymers facilitate the enrolment of the proteins to sites of DNA damage [4]. Inhibition of PARP-1 leads to the build-up of single-strand breaks (SSBs) that are converted to double strand-strand breaks (DSBs) during DNA replication. The produced DSBs can be repaired either by homologous recombination (HR) or non-homologous end joining (NHEJ) [5,6].Poly (ADP-ribose) polymerases (PARPs) are nuclear enzymes which catalyse the poly-ADP-ribosylation to association one or more ADP-ribose moieties from intracellular nicotinamide adenine dinucleotide (NAD+) covalently with target proteins [1–3]. The poly-ADP-ribosylation is generally involved in gene transcription, DNA damage repair, and cell-death signalling [4–6].

There are six domains in the structure of poly (ADP-ribose) polymerase 1 (PARP-1) protein revealed by current structural studies. Two of three zinc-binding domains have the function to detect and bind to DNA breaks and the third zinc-binding domain coordinates DNA-dependent enzyme activation [7]. The auto modification domain serves as acceptors of ADP-ribose moieties, which allow PARP-1 protein, mediated poly-ADP-ribosylation to it, and contains a BRCA1 C-terminus repeat motif [8–10]. The C-terminal catalytic domain catalyses the poly-ADP-ribosylation to combine one or more ADP-ribose moieties from intracellular nicotinamide adenine dinucleotide (NAD+) covalently with target proteins [11–13]. PARP-1 protein contains a DNA-binding domain, which can bind to DNA strand breaks and repair the damaged DNA over a low level, the inhibitors of poly(ADR-ribose) polymerase 1 (PARP-1) have been specified as the agents treated for cancer [14–17]. As Rucaparib is another PARP 1 and PARP 2 are the oral inhibitor that was tested in a phase I study with a constant daily dose range of 40–500 mg, as well as 240–840 mg BID. The subsequent recommended phase II dose of Rucaparib immunotherapy was 600 mg BID [Kristele it *et al.* 2014]. In a phase II trial of Rucaparib immunotherapy as advanced stage of g *BRCA* m of breast and ovarian cancers, Rucaparib was well accepted in doses of 480 mg daily, however aside from the recognition that its activity related with the platinum-free interval, no clearly defined phase II dose was established [Drew *et al.* 2016]. Rucaparib is a potent mammalian poly (ADP-ribose) polymerase (PARP) 1, 2 and 3 inhibitor with anticancer properties. PARP is an enzyme that plays a critical role in DNA repair by triggering response pathways and expediting repair [2], and defects in these repair mechanisms have been established in various malignancies, including cancer. Regulation of repair pathways is critical in encouraging necessary in cell death. BRCA genes are tumour suppressor genes mediate numerous cellular process including DNA replication, transcription regulation, cell cycle checkpoints, apoptosis, chromatin structuring and homologous recombination (HR). Homologous recombination deficiency (HRD), along with PARP inhibition, is a susceptibility that enhances the cell death pathway when the single mutations alone would certificate viability [13]. Ovarian cancer commonly retains the defects in DNA repair pathways such as HRD due to BRCA mutations or otherwise. There are three main types of ovarian cancer: epithelial (90%), germ cell (5%) and sex cord stromal cell (5%). Epithelial ovarian is the existence most common, fifth leading cause of cancer-related deaths in women in the United States [14, 12]. Advanced ovarian cancer particularly poses challenges due to reduced therapeutic response rates from standard platinum-based chemotherapy and overall survival rates [15].

Rucaparib has shown to induce cytotoxicity in tumour cell lines with insufficiencies in BRCA1/2 and other DNA repair genes [FDA Label]. Of all the BRCA1/2 mutations in ovarian cancer, most are due to germ line mutations (18%), and approximately 7% represent somatic mutations acquired within the tumour [4]. The indication of Rucaparib as an oral immunotherapy in patients with deleterious BRCA mutation (germ line and/or somatic) linked in advanced ovarian cancer was accelerated approval in 2016 for the selected patients who have previously received greater than two lines of platinum-based therapy [11]. It is currently marketed in the US under the brand name Rubraca that contains Rucaparib camsylate as an active ingredient. The identification of patients who are entitled for Rucaparib therapy is accomplished via in vitro diagnostic tests to detect the presence of a harmful BRCA mutation (germ line and/or somatic). The FDA-approved test qualitatively notices the sequence alterations in the BRCA1 and BRCA2 (BRCA1/2) genes. More information can be initiate on the FDA Website [9]. While Rucaparib is specified for deleterious BRCA mutation (germ line and/or somatic)-associated advanced ovarian cancer, there is proof that its antitumor activity is also clinically effective against ovarian tumours with high homologous recombination deficiency (HRD) loss of heterozygosis (LOH) [4, 16].

MATERIALS AND METHODS

PROTEIN AND LIGAND PREPARATION

The crystal structure of PARA–1 protein was retrieved by Protein data bank database (http://www.rcsb.org/). The Resolution of protein is2.3 Å and the Rucaparib drug was retrieved from Drug bank database (https://www.drugbank.ca/).

DESIGNING OF LEAD LIBRARY BY VIRTUAL SCREENING

The 2D structure of the chemical compound was retrieved from Zinc database (http://zinc.docking.org/) and the lead library was designed by using Marvin Sketch software 5.8.

DRUG LIKENESS SCREENING / BIOAVAILABILITY

The Molecular descriptors and the drug likeliness properties of the chemical compound were analysed using the tool Molinspriation server (http://www.molinspiration.com/), based on Lipinski's Rule of Five of molecular properties such as (logP, Polar surface area, number of hydrogen bond donors and acceptors), as well as predication of bioavailability score for the most important drug targets (GPCR ligand, Kinase inhibitors, ion channel modulators, enzyme and

nuclear receptor (Sathish Kumar Paramashivam *et al*. Usually molecules that meet the criteria for biological activity fulfil the characteristics contains in the Lipinski's Rule of Five (Lipinski *et al*. 1997).

PHARMACOKINETICS SCREENING BY ADMET PROPERTIES

The Pharmacokinetics properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the chemical compound. The Rucaparib has been predicated using admetSAR database (http://lmmd.ecust.edu.cn).

ACTIVE SITE PREDICATION

Active site of PARP-1 was identified using CASTp server (Computer Atlas of Surface Topology of protein) (Dundas *et al*. 2006) (http://sts.bioe.uic.edu/castp/) the program automatically locating and measuring protein pockets and cavities, is based on precise computational geometry method.

MOLECULAR DOCKING STUDIES

Based on the pharmacokinetics properties the compound was selected for docking studies. To validate the drug target compound the molecular docking was performed on active compound with screened compound by Auto dock vena 4.1 (http://vina.scripps.edu/) open-source program for doing molecular docking. It was designed and implemented by (Dr. Oleg Trott) was used for the screened chemical compound of selected ligand from Zinc database.

STRUCTURE VISUALIZATION

Based on the molecular docking of all 7 chemical compounds the docked molecule was visualized by using Pymol structure visualization tool.

RESULTS AND DISCUSSION

PROTEIN AND LIGAND PREPARATION

The protein PARP–1 ID 2RF5 was retrieved from Protein data bank and the chemical compound of Rucaparibwas retrieved from Drug bank database as shown in Fig. no 1.
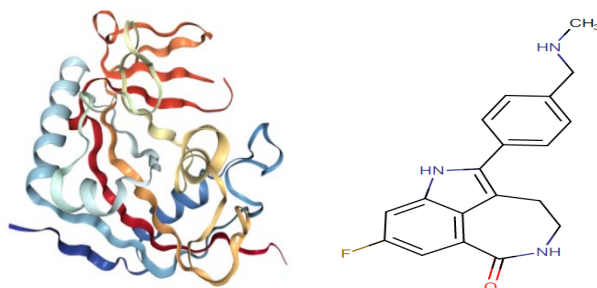
**Fig. no 1: A) Shows the 3D Structure of Protein B) Shows the
2D Structure of Chemical Structure of Ligand**

DESIGNING OF LEAD LIBRARY BY VIRTUAL SCREENING

Drug library of selected Rucaparib compound were designed manually by using by using Marvin Sketch server. For the chemical compound we ready-made 7 derivatives for Rucaparib based their functional group as shown in Fig. no 2.
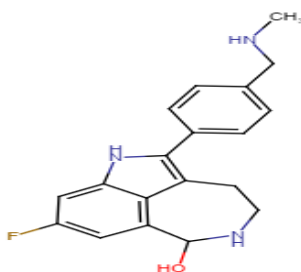
CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3C=C(F)CC(N1)C23
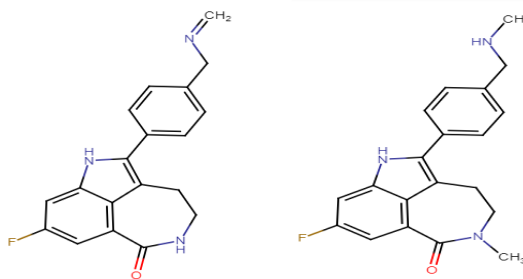
**FC1=CC2=C3C(NC(=C3CCNC2=O)C2=CC=C(CN=C)C=C2)=C1
CNCC1=CC=C(C=C1)C1=C2CCN(C)C(=O)C3=C2C(N1)=CC(F)=C3**
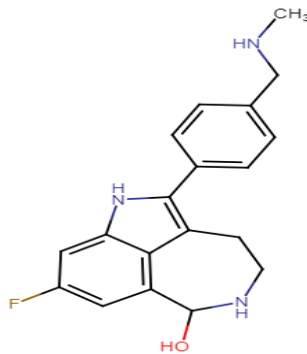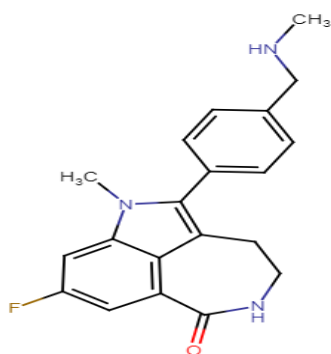
CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(=CC(F)=C3)N1C
CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3=C2C(N1)=CC(F)=C3
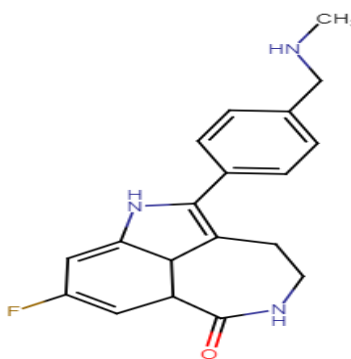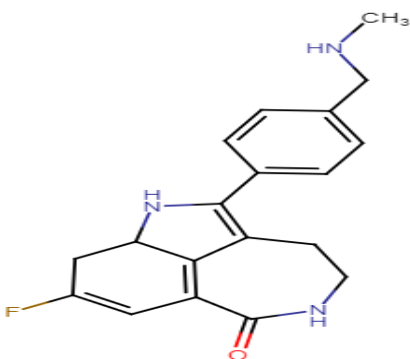
CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(CC(F)=C3)N1
CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3C=C(F)C=C(N1)C23

Fig. no 2: Analogs of Rucaparib Compound 7 Derivatives for Rucaparib and Best Binging Affinity Lead Compound

DRUG LIKENESS SCREENING / BIOAVAILABILITY

Rucaparib compound were analysed using the tool Molinspiration server, based on the Lipinski's Rule of Five. It shows the molecule number, SMILES, H–atom donor, H–atom acceptors, logp and molecular weight of all 7 molecules as shown in the Table 1.

Table 1: Drug Likeness Screening/ Bioavailability Were Analysed of Rucaparib Compound

Mol No	Smiles	H Atoms	H Donor	H Acceptor	Mi–Log	Mol. wt
1	CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3C=C(F)CC(N1)C23	24	4	3	2.52	325.39
2	FC1=CC2=C3C(NC(=C3CCNC2=O)C2=CC=C(CN=C)C=C2)=C1	24	4	2	2.71	321.36
3	CNCC1=CC=C(C=C1)C1=C2CCN(C)C(=O)C3=C2C(N1)=CC(F)=C3	24	4	2	2.40	337.40
4	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(=CC(F)=C3)N1C	25	4	2	2.69	337.40
5	CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3=C2C(N1)=CC(F)=C3	24	4	4	2.52	325.39
6	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(CC(F)=C3)N1	24	4	3	1.60	325.39
7	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3C=C(F)C=C(N1)C23	24	4	3	2.31	325.39

PHARMACOKINETICS SCREENING BY ADMET PROPERTIES

The Pharmacokinetics properties of the compound Rucaparib can be predicated using admet SAR database and it gives the pharm logical properties by using admet SAR database we predicated the BBB+ (Blood Brain Barrier), HIA+ (Human Intestinal Absorption), Caco2+, ADMET Toxicity and Carcinogens of all 7 molecule as shown in Table no 2

Table 2: Admet Properties for Rucaparib Compound

Mol No.	Smiles	BBB+	HIA+	CaCo2+	Admet Toxicity	Carcinogens
1	CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3C=C(F)CC(N1)C23	0.9661	0.9973	0.5989	0.7131	0.6544
2	FC1=CC2=C3C(NC(=C3CCNC2=O)C2=CC=C(CN=C)C=C2)=C1	0.9939	0.9897	0.5677	0.7665	0.6582
3	CNCC1=CC=C(C=C1)C1=C2CCN(C)C(=O)C3=C2C(N1)=CC(F)=C3	0.9736	1.0000	0.5868	0.7159	0.6967
4	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(=CC(F)=C3)N1C	0.9770	1.0000	0.5308	0.7074	0.6663
5	CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3=C2C(N1)=CC(F)=C3	0.9661	0.9973	0.5989	0.7131	0.6544
6	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(CC(F)=C3)N1	0.9843	1.0000	0.5714	0.6173	0.5857
7	CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3C=C(F)C=C(N1)C23	0.9855	1.0000	0.5653	0.6129	0.5741

ACTIVE SITE PREDICATION

Byusing CASTp (Compound Atlas of Surface Topology of Proteins) server it gives 31 pockets for particular protein of interest 2RF5 and the highlighted residues shows interacting residue with desired pockets in the chain A as shown in Fig. no 3. From the residue number 1186 to 1248 residues it shows the number of the residues and atoms involved in the pockets as shown in the Table no 3.

Sequence

Chain A

M H H H H H H S S G V D L G T E N L Y F Q S M Q G T N P Y L T F H C V N Q G T I L L D L A P E D K E Y Q S V E E E M Q S T I R E H
R D G G N A G G I F N R Y N V I R I Q K V V N K K L R E R F C H R Q K E V S E E N H N H H N E R M L F H G S P F I N A I I H K G F
D E R H A Y I G G M F G A G I Y F A E N S S K S N Q Y V Y G I G G G T G C P T H K D R S C Y I C H R Q M L F C R V I L G K S F L Q
F S T I K M A H A P P G H H S V I G R P S V N G L A Y A E Y V I Y R G E Q A Y P E Y L I T Y Q I M K P E A P S Q T A T A A E Q

Features

Feature	Position(s)	Description	Reference
metal ion-binding site	A: 1234	Zinc	Uniprot: O95271
metal ion-binding site	A: 1237	Zinc	Uniprot: O95271
metal ion-binding site	A: 1242	Zinc	Uniprot: O95271
metal ion-binding site	A: 1245	Zinc	Uniprot: O95271
mutagenesis site	A: 1184	H->A. Loss of activity; when associated with A-1291.	Uniprot: O95271
mutagenesis site	A: 1291	E->A. Loss of activity; when associated with A-1184.	Uniprot: O95271

Fig. no 3: Shows in Pockets in the Chain A

Table 3: Shows the Number of the Residues and Atoms Involved in the Pockets

POCID	Chain	SEQID	AA	Atom
1	A	1187	PRO	C
1	A	1187	PRO	O
1	A	1187	PRO	CB
1	A	1189	ILE	N
1	A	1189	ILE	CB
1	A	1189	ILE	CG1
1	A	1190	ASN	N
1	A	1190	ASN	CB

POCID	Chain	SEQID	AA	Atom
1	A	1225	VAL	O
1	A	1225	VAL	CG1
1	A	1226	TYR	CA
1	A	1226	TYR	CD1
1	A	1226	TYR	CE1
1	A	1226	TYR	OH
1	A	1235	PRO	CG
1	A	1235	PRO	CD
1	A	1246	HIS	O
1	A	1247	ARG	CA
1	A	1248	GLN	N
1	A	1248	GLN	O
1	A	1248	GLN	CB
1	A	1248	GLN	CG
1	A	1248	GLN	OE1

MOLECULAR DOCKING STUDIES

Docking is a computational method which predicts the preferred orientation of molecule by using Auto Dock Vina in PyRx virtual screening tool (Wolf, 2009; Trott and Olson, 2010). As the screened chemical compound were docked into active site of refined model. For the docking of ligand into target protein binging pocket (Sousa *et al.*, 2006) and to estimate the binding affinities of docked ligand. The docked conformation to the lowest binding energy was selected as the most probable binding conformation. The total screened compound was 7 docked against PARP-1 of active site. The best 5 virtual screened chemical compound showed best binding energies and significant energies with target protein as shown in Table no 4.

Table 4: Shows Protein and Ligand Binding Energy Values

Sr. No.	Protein–Ligand	Binding Affinities (K.Cal/Mol)
1	2RF5-CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3C=C(F)CC(N1)C23	-3.53
2	2RF5-FC1=CC2=C3C(NC(=C3CCNC2=O)C2=CC=C(CN=C)C=C2)=C1	-3.89
3	2RF5-CNCC1=CC=C(C=C1)C1=C2CCN(C)C(=O)C3=C2C(N1)=CC(F)=C3	-4.74
4	2RF5-NCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(=CC(F)=C3)N1C	-5.36
5	2RF5-CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3=C2C(N1)=CC(F)=C3	-6.10
6	2RF5-CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3=C2C(CC(F)=C3)N1	-4.07
7	2RF5-CNCC1=CC=C(C=C1)C1=C2CCNC(=O)C3C=C(F)C=C(N1)C23	-5.69

STRUCTURE VISUALIZATION

The docked target protein and lead compound was visualized using Pymol it shows binding residue, hydrogen bond, number of residue interacting against the best

docking compound **CNCC1=CC=C(C=C1)C1=C2CCNC(O)C3=C2C(N1)=CC(F)=C3** as shown in the Fig. no 4.
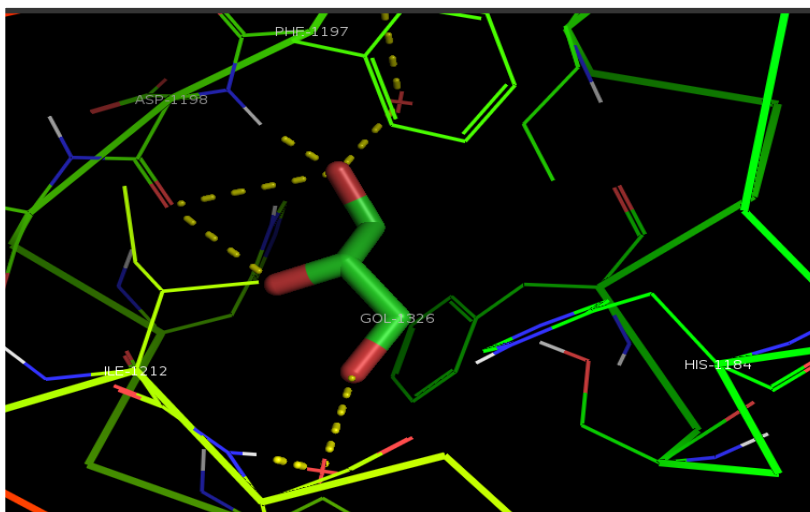
Fig. 4: Graphical View of Best Binding Protein and Lead Compound Interaction

CONCLUSION

The DNA repair system involving the PARP-1 and PARP2 and play an important role normal development of the organism. The Molecular mechanisms in the ovarian cancer pathogenesis identify numerous targeted agents that are now entering in the clinical practice. Nowadays, the family of poly (ADP-ribose) polymerase (PARP) inhibitors represents as a widely examined and promising alternative for the targeted therapy of ovarian malignancies. PARP inhibitors abuse the synthetic lethality concept to inhibit the DNA damage repair, causing cancer cell death.

Many new approaches to anticancer drug discovery are now under evaluation, as reviewed here. Rucaparib is a derived drug for ovarian cancer. Recent increase in the area suggested that if support for anticancer drug discovery is adequate, new approach should lead to the development of valuable new strategies for anticancer therapy in the near future. In the present study, we derived a chemical compound and molecular docking was done, based on that the Rucaparib and its virtual screened compound done the compound shows the binding energy with desired protein molecule is 6.10 B.E is 6-fluoro-2-{4-[(methylamino) methyl] phenyl}-3,10-diazatricyclo[6.4.1.0^{4,13}]trideca-1,4,6,8(13)-tetraen-9-ol and we are moving for the synthesis of compound.

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Identification of Ovarian Cancer Mutations using Next Generation Sequencing

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ABSTRACT—*Ovarian cancer is one of the most common cancers and cause of cancer deaths in women. This is because; with conventional sequencing technologies, ovarian cancer has been detected at advanced stage. But now, with the advent of Next-generation sequencing (NGS), researchers can identify all possible cancer mutations which were fail to identify using conventional sequencing technologies like Sanger sequencing. With NGS, sequencing of genes of cancer patients can be performed at lower cost and with higher throughput. Thus, it will help in early disease diagnosis of ovarian cancer. NGS is implemented mainly for whole-genome, whole-exome and transcriptome sequences. There are various NGS tools such as BWA, Bowtie, Galaxy, VarScan, ANNOVAR etc. are used for performing computational analysis of NGS reads which are generated from laboratory NGS platforms like Illumina, Roche, ABI/ SOLID etc. The present comprehensive research encompasses alignment of NGS reads of ovarian cancer patients, identification, annotation and visualization of identified ovarian cancer mutations. These identified mutations will have clinical significance and will play an eminent role in target identification of ovarian cancer. In the field of clinical oncology, NGS has already proven and proving its potency in disease diagnosis because of which the mortality rate has been reduced.*

KEYWORDS: *Next Generation Sequencing, Mutations, Ovarian Cancer, Sanger Sequencing, Variant Identification*

INTRODUCTION

Ovarian Cancer is one of the global leading causes of cancer death. This is due to detection of cancer at advanced stage [1]. There are 3 types of ovarian cancer: Epithelial tumors, Germ cell tumors and Stromal tumors. About 85% to 90% of ovarian cancers fall under malignant ovarian epithelial tumours, known as epithelial ovarian carcinomas [2]. The advancement of Next Generation Sequencing (NGS) has given a promising way to identify disease specific mutations (Stephan Pabinger, 2013). With NGS, researchers can identify all possible range of mutations from genes at faster rate and lower cost as compared to previous sequencing technologies such as Sanger sequencing. NGS can be implemented mainly for 3 types of sequencing: Whole exome sequencing, Whole genome sequencing and Transcriptome sequencing. NGS has been used to sequence human genomes and cancer genomes from which mutations of different cancer types have been identified. Along with these cancer molecular diagnosis [4, 5] and prognosis [6, 7], NGS has applications in prenatal diagnosis [8], medical genetics and pharmacogenomics [9], molecular diagnosis of genetic disease [10] and infectious disease [11, 12].

Three commonly used platforms for performing Next generation sequencing are three Roche 454, llumina and ABI Solid. After generation of raw sequence data from lab work with these platforms, it will be analyzed computationally using different NGS tools. The computational analysis of raw NGS data involves different steps: 1. Quality assessment of the raw NGS data, 2. Alignment of NGS reads to a reference genome 3. Identification of variants 4. Annotation of the variants and 5. Visualization of Data. Various NGS tools are used for performing these steps such as Bowtie, BWA etc. are used for performing alignment of NGS reads, VarScn2, CRISP, GATK etc. are used for variant identification, ANNOVAR, Ann Tools, snp Eff etc. are used for variant annotation, Ensembl genome browser, UCSC genome browser, VEGA genome browser etc. are used for variant visualization [3].

The current NGS research is focused on computational analysis of NGS reads of Ovarian cancer patients retrieved from Sequence Read Archive, the cancer mutations were then identified using VarScan, among total 173 identified mutations, 3 mutations are damaging mutations which were annotated and Visualized using Variant Effet Predictor.

MATERIALS AND METHODS

RETRIEVAL OF NGS READS

Total 10 NGS reads of ovarian cancer patients were retrieved from sequence read archive. The reads can be retrieved either by executing linux commands for NGS or by Aspera connect toolkit.

ALIGNMENT WITH REFERENCE GENOME

The retrieved NGS reads were then aligned to reference genome hg19 using BWA (Burrows Wheeler Aligner) tool. For that, reference index of hg19 was created and BAM (Binary Alignment/Map) files of aligned NGS reads were generated.

IDENTIFICATION OF MUTATIONS

Ovarian cancer mutations were identified using VarScan tool. For that, BAM file of aligned NGS reads was given as input to VarScan. Mutations were identified by setting standard parametric values for min-coverage, min-var-freq and p-value. For identification of SNP, the values were set as, min-coverage=10, min-var-freq=0.20 and p-value=0.05. For identification of INDEL, the values were set as, min-coverage=10, min-var-freq=0.10 and p-value=0.10.

ANNOTATION AND VISUALIZATION OF MUTATIONS

The identified ovarian cancer mutations were then annotated and visualized using Variant Effect Predictor of Ensembl genome browser. For that vcf (variant call format) file of identified mutations was given as input.

RESULTS AND DISCUSSION

Total 173 mutations were identified using VarScan tool. Among that, 63 mutations were found SNPs, listed in Table 2 and 110 mutations were INDELS listed in Table 1, which were annotated and visualized with Variant Effect Predictor of Ensembl with annotated features such as Location, Allele, Consequence, Impact, Symbol, Gene, Feature type, Feature, Biotype etc. Among 173 mutations, 3 mutations were found damaging mutations with PolyPhen annotation, which can be probable disease causing, as depicted in Table 3.

Similarly, Tom Walsh *et al*. [13] were identified 21 inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Magdalena Koczkowska *et al*. [14] was detected 27 somatic BRCA1/2 mutations of ovarian cancer using BRCA Tumor MASTR Plus assay. As compared with these results, more number of ovarian cancer mutations was identified in this study using computational analysis of NGS data of ovarian cancer patients. The identified mutations will have biological significance and will be useful for target identification in drug discovery.

Table 1: Mutations with Indels

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
1:91852832-91852832	C	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000212 355	protein_codi ng
1:91852832-91852832	C	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000370 399	protein_codi ng
1:91852832-91852832	C	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000417 833	protein_codi ng
1:91852832-91852832	C	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000465 892	protein_codi ng
1:91852832-91852832	C	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000525 962	protein_codi ng
1:91852832-91852832	C	intron_variant, NMD_transcript _variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000532 540	nonsense_m ediated_deca y
1:91852832-91852832	C	intron_variant, NMD_transcript _variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000533 089	nonsense_m ediated_deca y
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000212 355	protein_codi ng
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000370 399	protein_codi ng

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000417 833	protein_codi ng
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000465 892	protein_codi ng
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000525 962	protein_codi ng
1:91853013-91853013	TC	intron_variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000532 540	nonsense_m ediated_deca y
1:91853013-91853013	TC	intron_variant, NMD_transcript _variant	Modifier	TGFBR3	ENSG000000 69702	Transcript	ENST00000533 089	nonsense_m ediated_deca y
1:228750511-228750511	A	downstream_gen e_variant	Modifier	RHOU	ENSG000001 16574	Transcript	ENST00000366 691	protein_codi ng
1:228752752-228752752	A	intergenic_varia nt	Modifier	-	-	Transcript	-	-
1:228754993-228754993	A	intergenic_varia nt	Modifier	-	-	Transcript	-	-
1:228757208-228757208	A	intergenic_varia nt	Modifier	-	-	-	-	-
1:228759428-228759428	A	intergenic_varia nt	Modifier	-	-	-	-	-
1:228761670-228761670	A	intergenic_varia nt	Modifier	-	-	-	-	-
1:228768392-228768392	A	intergenic_varia nt	Modifier	-	-	-	-	-
1:228770632-228770632	A	intergenic_varia nt	Modifier	-	-	-	-	-
1:228772857-228772857	A	upstream_gene_ variant	Modifier	AL07862 4.2	ENSG000002 71475	Transcript	ENST00000605 063	processed_ps eudogene
1:228775098-228775098	A	upstream_gene_ variant	Modifier	AL07862 4.2	ENSG000002 71475	Transcript	ENST00000605 063	processed_ps eudogene
1:228781801-228781801	A	downstream_gen e_varian	Modifier	AL07862 4.2	ENSG000002 71475	Transcript	ENST00000605 063	processed_ps eudogene
1:228781801-228781801	A	regulatory_regio n_variant	Modifier	-	-	Regulatory Feature	ENSR00000021 355	open_chrom atin_region
2:133038353-133038353	A	intron_variant	Modifier	NCKAP5	ENSG000001 76771	Transcript	ENST00000317 721	protein_codi ng
2:133038353-133038353	A	intron_variant	Modifier	NCKAP5	ENSG000001 76771	Transcript	ENST00000405 974	protein_codi ng
2:133038353-133038353	A	intron_variant	Modifier	NCKAP5	ENSG000001 76771	Transcript	ENST00000409 213	protein_codi ng
2:133038353-133038353	A	intron_variant	Modifier	NCKAP5	ENSG000001 76771	Transcript	ENST00000409 261	protein_codi ng
21:9827449-9827449	T	regulatory_regio n_variant	Modifier	-	-	Regulatory Feature	ENSR00000299 600	CTCF_bindi ng_site
21:9827449-9827449	T	intron_variant	Modifier	-	-	-	-	-
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000263 962	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000392 649	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000463 761	protein_codi ng

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000464 614	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000465 751	protein_codi ng
3:179879685-179879685	A	intron_variant, non_coding_tran script_variant	Modifier	PEX5L-AS1	ENSG000002 43799	Transcript	ENST00000466 064	antisense_R NA
3:179879685-179879685	A	intron_variant, non_coding_tran script_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000467 440	processed_tr anscript
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000467 460	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000468 741	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000469 198	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000472 994	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000476 138	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000485 199	protein_codi ng
3:179879685-179879685	A	downstream_gen e_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000487 198	processed_tr anscript
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000491 640	protein_codi ng
3:179879685-179879685	A	intron_variant	Modifier	PEX5L	ENSG000001 14757	Transcript	ENST00000496 721	protein_codi ng
5:71146929-71146930	-	intergenic_varia nt	Modifier	-	-	-	-	-
5:71146932-71146932	T	intergenic_varia nt	Modifier	-	-	-	-	-
6:4428300-4428300	T	downstream_gen e_variant	Modifier	RNA5SP2 02	ENSG000002 01185	Transcript	ENST00000364 315	rRNA
6:120583487-120583487	T	intergenic_varia nt	Modifier	-	-	-	-	-
:120583490-120583491	-	intergenic_varia nt	Modifier	-	-	-	-	-
:153741660-153741660	T	intergenic_varia nt	Modifier	-	-	-	-	-
7:128337576-128337576	T	intron_variant	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000223 073	protein_codi ng
7:128337576-128337576	T	intron_variant	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000415 472	protein_codi ng
7:128337576-128337576	T	downstream_gen e_varian	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000459 726	protein_codi ng
7:128337576-128337576	T	intron_variant	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000478 061	protein_codi ng
7:128337576-128337576	T	upstream_gene_ variant	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000487 602	nonsense_m ediated_deca y
7:128337576-128337576	T	upstream_gene_ variant	Modifier	RBM28	ENSG000001 06344	Transcript	ENST00000488 249	retained_int ron

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
Un_gl000220: 109488-109488	T	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 109488-109488	T	downstream_gen e_variant	Modifier	pRNA	ENSG000002 76197	Transcript	ENST00000619 317	misc_RNA
Un_gl000220: 109522-109522	T	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 109522-109522	T	downstream_gen e_variant	Modifier	Prna	ENSG000002 76197	Transcript	ENST00000619 317	misc_RNA
Un_gl000220: 110035-110035	GT	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 110035-110035	GT	downstream_gen e_variant	Modifier	pRNA		Transcript	ENST00000619 317	misc_RNA
Un_gl000220: 110036-110036	T	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 110036-110036	T	downstream_gen e_variant	Modifier	pRNA	ENSG000002 76197	Transcript	ENST00000619 317	misc_RNA
Un_gl000220: 110321-110321	G	upstream_gene_variant	MODIFI ER	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 110321-110321	G	downstream_gen e_variant	Modifier	pRNA	ENSG000002 76197	Transcript	ENST00000619 317	misc_RNA
Un_gl000220: 110321-110321	G	TF_binding_site _variant	Modifier	-	-	MotifFeatu re	MA0099.2	-
Un_gl000220: 110491-110492	-	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 110491-110492	-	TF_binding_site _variant	Modifier	-	-	MotifFeatu re	MA0016.1	-
Un_gl000220: 110691-110691	T	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 114076-114076	T	downstream_gen e_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 114087-114088	-	downstream_gen e_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 114087-114088	-	TF_binding_site _variant	Modifier	-	-	MotifFeatu re		-
Un_gl000220: 114665-114665	T	downstream_gen e_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA

[33]

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
Un_gl000220: 116104-116105	-	downstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 116110-116110	GG	downstream_gene_variant	MODIFI ER	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 116156-116156	T	downstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 116157-116157	G	downstream_gene_variant	Modifier	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 116295-116295	T	downstream_gene_variant	MODIFI ER	RNA5-8SN2	ENSG000002 74917	Transcript	ENST00000611 446	rRNA
Un_gl000220: 117823-117823	T	intergenic_varia nt	Modifier	-	-	-	-	-
Un_gl000220: 153460-153460	T	downstream_gene_variant	Modifier	pRNA	ENSG000002 73937	Transcript	ENST00000614 535	misc_RNA
Un_gl000220: 153460-153460	T	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 153494-153494	T	downstream_gene_variant	Modifier	pRNA	ENSG000002 73937	Transcript	ENST00000614 535	misc_RNA
Un_gl000220: 153494-153494	T	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 154007-154007	GT	downstream_gene_variant	Modifier	pRNA	ENSG000002 73937	Transcript	ENST00000614 535	misc_RNA
Un_gl000220: 154007-154007	GT	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 154008-154008	T	downstream_gene_variant	Modifier	pRNA	ENSG000002 73937	Transcript	ENST00000614 535	misc_RNA
Un_gl000220: 154008-154008	T	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 154293-154293	G	downstream_gene_variant	Modifier	pRNA	ENSG000002 73937	Transcript	ENST00000614 535	misc_RNA
Un_gl000220: 154293-154293	G	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 154293-154293	G	TF_binding_site _variant	Modifier	-	-	MotifFeatu re	MA0099.2	-
Un_gl000220: 154663-154663	T	upstream_gene_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
Un_gl000220: 154664-154664	TG	upstream_gene_varian	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 157712-157712	G	downstream_gen e_varian	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 158048-158048	T	downstream_gen e_varian	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 158058-158060	-	downstream_gen e_varian	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 158058-158060	-	TF_binding_site _variant	Modifier	-	-	MotifFeatu re	MA0470.1	-
Un_gl000220: 158637-158637	T	downstream_gen e_varian	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 158812-158812	T	downstream_gen e_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 159342-159342	T	downstream_gen e_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 160082-160082	GG	downstream_gen e_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 160128-160128	T	downstream_gen e_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA
Un_gl000220: 160129-160129	G	downstream_gen e_variant	Modifier	RNA5-8SN3	ENSG000002 73730	Transcript	ENST00000619 779	rRNA

Table 2: Mutations with SNPS

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
12:127650639 -127650639	G	regulatory_region _variant	Modifier	-	-	RegulatoryFeature	ENSR00 00005916 8	open_chro matin_reg ion
2:203211034-203211034	G	missense_variant	Modifier	NBEAL 1	ENSG00 00014442 6	Transcript	ENST00 00041457 6	protein_co ding
2:203211034-203211034	G	missense_variant	Modifier	NBEAL 1	ENSG00 00014442 6	Transcript	ENST00 00043446 9	protein_co ding
2:203211034-203211034	G	missense_variant	Modifier	NBEAL 1	ENSG00 00014442 6	Transcript	ENST00 00044980 2	protein_co ding
12:38555303-38555303	A	intron_variant, non_coding_tran script_variant	Modifier	AC08789 7.2	ENSG007 00025811 9	Transcript	ENST00 00055263 9	lincRNA
17:8076802-8076802	A	intron_variant	Modifier	ALOX12 B	ENSG00 00017947 7	Transcript	ENST00 00031914 4	protein_co ding

[35]

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
17:8076802-8076802	A	upstream_gene_variant	Modifier	AC12949 2.1	ENSG00 00021499 9	Transcript	ENST00 00039941 3	antisense_ RNA
17:8076802-8076802	A	intron_variant, non_coding_tran script_variant	Modifier	ALOX12 B	ENSG00 00017947 7	Transcript	ENST00 00057735 1	processed_ transcript
17:8076802-8076802	A	intron_variant, non_coding_tran script_variant	Modifier	ALOX12 B	ENSG00 00017947 7	Transcript	ENST00 00058327 6	retained_i ntron
17:8076802-8076802	A	intron_variant, non_coding_tran script_variant	Modifier	ALOX12 B	ENSG00 00017947 7	Transcript	ENST00 00058411 6	retained_i ntron
17:8076802-8076802	A	downstream_gen e_variant	Modifier	AC12949 2.6	ENSG00 00027501 1	Transcript	ENST00 00061138 3	unprocess ed_pseudo gene
1:228768400-228768400	A	intergenic_varian t	Modifier	-	-	-	-	-
12:127650639 -127650639	G	intergenic_varian t	Modifier	-	-	-	-	-
7:128337580-128337580	A	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00022307 3	protein_co ding
7:128337580-128337580	A	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00041547 2	protein_co ding
7:128337580-128337580	A	downstream_gen e_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00045972 6	protein_co ding
7:128337580-128337580	A	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00047806 1	protein_co ding
7:128337580-128337580	A	upstream_gene_v ariant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00048760 2	nonsense_ mediated_ decay
7:128337580-128337580	A	upstream_gene_v ariant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00048824 9	retained_i ntron
7:128337581-128337581	T	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00022307 3	protein_co ding
7:128337581-128337581	T	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00041547 2	protein_co ding
7:128337581-128337581	T	downstream_gen e_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00045972 6	protein_co ding
7:128337581-128337581	T	intron_variant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00047806 1	protein_co ding
7:128337581-128337581	T	upstream_gene_v ariant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00048760 2	nonsense_ mediated_ decay
7:128337581-128337581	T	upstream_gene_v ariant	Modifier	RBM28	ENSG00 00010634 4	Transcript	ENST00 00048824 9	retained_i ntron

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
8:70602561-70602561	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000262213	protein_coding
8:70602561-70602561	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000518678	protein_coding
8:70602561-70602561	C	intron_variant, non_coding_transcript_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000520700	processed_transcript
8:70602561-70602561	C	intron_variant, non_coding_transcript_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000521049	processd_transcript
8:70602561-70602561	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000521425	protein_coding
8:70602562-70602562	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000262213	protein_coding
8:70602562-70602562	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000518678	protein_coding
8:70602562-70602562	C	intron_variant, non_coding_transcript_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000520700	processed_transcript
8:70602562-70602562	C	intron_variant, non_coding_transcript_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000521049	processed_transcript
8:70602562-70602562	C	intron_variant	Modifier	TRAM1	ENSG00000067167	Transcript	ENST00000521425	protein_coding
Un_gl000220:110039-110039	G	upstream_gene_variant	Modifier	RNA5-8SN2	ENSG00000274917	Transcript	ENST00000611446	rRNA
Un_gl000220:110039-110039	G	downstream_gene_variant	Modifier	pRNA	ENSG00000276197	Transcript	ENST00000619317	misc_RNA
Un_gl000220:110046-110046	C	upstream_gene_varian	Modifier	RNA5-8SN2	ENSG00000274917	Transcript	ENST00000611446	rRNA
Un_gl000220:110046-110046	C	downstream_gene_variant	Modifier	pRNA	ENSG00000276197	Transcript	ENST00000619317	misc_RNA
Un_gl000220:110260-110260	G	upstream_gene_varian	Modifier	RNA5-8SN2	ENSG00000274917	Transcript	ENST00000611446	rRNA
Un_gl00020:110260-110260	G	downstream_gene_variant	Modifier	pRNA	ENSG00000276197	Transcript	ENST00000619317	misc_RNA
Un_gl000220:110695-110695	G	upstream_gene_varian	Modifier	RNA5-8SN2	ENSG00000274917	Transcript	ENST00000611446	rRNA
Un_gl000220:110756-110756	C	upstream_gene_varian	Modifier	RNA5-8SN2	ENSG00000274917	Transcript	ENST00000611446	rRNA

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
Un_gl000220 :115228- 115228	A	downstream_gen e_variant	Modifier	RNA5- 8SN2	ENSG00 00027491 7	Transcript	ENST00 00061144 6	rRNA
Un_gl000220 :115908- 115908	T	TF_binding_site _variant	Modifier	-	-	MotifFeature	MA0527. 1	-
Un_gl000220 :115375- 115375	G	downstream_gen e_variant	Modifier	RNA5- 8SN2	ENSG00 00027491 7	Transcript	ENST00 00061144 6	rRNA
Un_gl000220 :115908- 115908	T	downstream_gen e_variant	Modifier	RNA5- 8SN2	ENSG00 00027491 7	Transcript	ENST00 00061144 6	rRNA
Un_gl000220 :154011- 154011	G	downstream_gen e_variant	Modifier	pRNA	ENSG00 00027393 7	Transcript	ENST00 00061453 5	misc_RN A
Un_gl000220 :154011- 154011	G	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :154018- 154018	C	downstream_gen e_varian	Modifier	pRNA	ENSG00 00027393 7	Transcript	ENST00 00061453 5	misc_RN A
Un_gl000220 :154018- 154018	C	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :118253- 118253	T	intergenic_varian t	Modifier	-	-	-	-	-
Un_gl000220 :154232- 154232	G	downstream_gen e_variant	Modifier	pRNA	ENSG00 00027393 7	Transcript	ENST00 00061453 5	misc_RN A
Un_gl000220 :154232- 154232	G	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	Rrna
Un_gl000220 :154667- 154667	G	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :154716- 154716	A	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :154728- 154728	C	upstream_gene_v ariant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :159274- 159274	C	downstream_gen e_variant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA
Un_gl000220 :159880- 159880	T	TF_binding_site _variant	Modifier	-	-	MotifFeature	MA0527. 1	-
Un_gl000220 :159277- 159277	C	downstream_gen e_variant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	Rrna
Un_gl000220 :159880- 159880	T	downstream_gen e_variant	Modifier	RNA5- 8SN3	ENSG00 00027373 0	Transcript	ENST00 00061977 9	rRNA

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype
X:74839738-74839738	G	intron_variant	Modifier	NEXMI F	ENSG00 00005003 0	Transcript	ENST00 00005568 2	protein_co ding
X:74839738-74839738	G	intron_variant	Modifier	NEXMI F	ENSG00 00005003 0	Transcript	ENST00 00061620 0	protein_co ding

Table 3: Damaging Mutations

Location	Allele	Consequence	Impact	Symbol	Gene	Feature Type	Feature	Biotype	Sift	Polyphe n
2:20321103 4-203211034	G	missense_varia nt	Modifier	NBEAL1	ENSG0 0000144 426	Transcript	ENST000 00414576	protein_co ding	0.52	0.579
2:20321103 4-203211034	G	missense_varia nt	Modifier	NBEAL1	ENSG0 0000144 426	Transcript	ENST000 00434469	protein_co ding	0.44	0.714
2:20321103 4-203211034	G	missense_varia nt	Modifier	NBEAL1	ENSG0 0000144 426	Transcript	ENST000 00449802	protein_co ding	0.47	0.987

CONCLUSION

Next generation sequencing has given a faster way to identify novel and rare cancer mutations at lower cost. With this study, the identified ovarian cancer mutations will be helpful for target identification in drug discovery process. NGS has solved the problem of diagnosis at advanced stage of Ovarian cancer and thus has reduced the mortality rate. Computational analysis of NGS data has given a potential insight into the field of Clinical oncology. In future, focus will be on computational analysis of more number of NGS reads to identify clinically significant Ovarian cancer mutations.

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Synthesis, Biological Evaluation and Molecular Modeling Studies of Novel Chromone/ Aza-Chromone Fused α-Aminophosphonates as SRC Kinase Inhibitors

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INTRODUCTION

Protein tyrosine kinases (PTKs) are enzymes that catalyze phosphorylation of tyrosine in many proteins through the transfer of the γ-phosphoryl group from ATP[1]. The Src family kinases (SFKs) comprises of nine different PTKs including c-Src, c-Yes, Fyn, Lck, Lyn, Hck, Frk, Blk and c-Fgr [1b, 2]. SFKs play important roles in the regulation of a wide variety of normal cellular signal transduction pathways, such as cell division, growth factor signaling differentiation, survival, adhesion, migration and invasion[3]. Src tyrosine kinase overexpression is regularly implicated in number of cancer diseases, such as colon, lung, breast, prostate, ovary and pancreas. Boustinib, dasatinib, and ponatinib are some of the Src/multikinase inhibitors that have been approved by the FDA for the treatment of chronic myelogenous leukemia and few others are in clinical trial for a variety of solid tumors[4] Hence, there has been raising interest in the development of Src kinase inhibitors in recent years, both for their use as research probes, delineating the specific functions of these kinases, and as potential anticancer/related therapeutics.

Chromone is well recognized as a privileged motif, due to its abundance in various natural products with wide range of biological properties that include anti-bacterial, antifungal, anti-cancer, anti-HIV and anti-ulcer agents[5]. Similarly, α-aminophosphonates and α-aminophosphonicacids constitute an important group of

naturally occurring compounds that are considered as isoelectronic analogues of the natural α-aminoacids. Owing to their structural similarity to ubiquitous phosphate esters and carboxylic acids coupled with inherent stability of the C–P bond, these phosphonates often display potent activities as enzyme inhibitors[6]. Further, they are well known for their biological activities such as anticancer, antiviral, antifungal and anti-leishmanial[7].
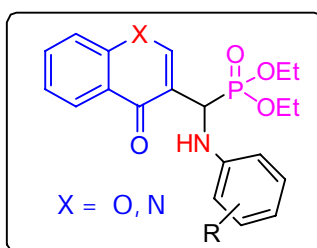

Fig. 1: Design of Chromone/ Azachromone Fused α-amino Phosphonate Conjugates

Recently, coumarin and chromone derivatives have also been evaluated for their Src kinase inhibition[8]. However, chromone/azachromone fused α-aminophosphonate conjugates have not been studied as Src kinase inhibitors. In this context, and in view of our long-standing interest in the chemistry of privileged chromone motif for various biological applications[9], in the present study, we report the synthesis of chromone/azachromone fused α-amino phosphonate conjugates. This study also provides insights on the structure activity relationship as well as the Src Kinase interaction with these compounds using molecular modeling and molecular dynamics simulation studies.

METHODOLOGY

PREPARATION OF MACROMOLECULE

The protein targets retrieved from RCSB Protein Data Bank were unphosphorylated proto-oncogenic tyrosine protein kinase Src (PDB code 1Y57) and phosphorylated tyrosine protein kinase Src (PDB code 2H8H) which served as docking receptors. The proteins were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v10.3[10]. The bond orders were assigned to residues, hydrogen atoms were added at pH 7.0. Minimization was carried out using OPLS 2005 force field with a RMSD cutoff value of 0.3Å.

PREPARATION OF LIGANDS

The 2D structures of all compounds i.e. 4c, 4i, 4j, staurosporine and PP2 were drawn and analyzed by Marvin view. The compounds were converted to 3D structure (.pdb) using LigPrep tool[11]. LigPrep is a Schrödinger suite tool which is

used to generate 3D structures from 2D structures, search tautomers, isomers for compounds and carry out energy minimization by applying the OPLS 2005 force field.

MOLECULAR DOCKING

The molecular docking was performed and analyzed via the Glide v6.8 docking tool[12]. The receptor grid was centered based on the active site of the protein using receptor grid generation tool. Ligands prepared using LigPrep were flexibly docked in grid box using Monte Carlo based simulation algorithm. An extra precision (XP) method was employed that generated binding poses based on energy. The favourably docked molecules were ranked according to the Glide Score.

MOLECULAR DYNAMICS SIMULATION

The docked c-Src kinase proteins with compound 4j were subjected to molecular dynamics simulation with the CHARMM 36 force field in GROMACS 4.6 tool[13]. The simulation was minimized using 5000 steps of Steepest Descent method. The system was later equilibrated at 300 Kelvin temperature and 1.06 pressures for 200 ps. The timestep set was 0.2 fs. The final production was run for 20ns and the distance, RMSF and hydrogen bond trajectories were calculated for the protein-ligand system.

STRUCTURE ACTIVITY CORRELATION MODELS

The 2D descriptors and Principal components were computed in MOE CCG computing package. The SVR model was developed using the RapidMiner data mining program[14]. The construction, validation of the SVR model was partitioned into three subsets in 70:20:10 ratio. The SVR model was built by employing the ε-SVR algorithm and radial basis function (RBF) kernel function, and validated using five-fold validation technique. The algorithm utilizes three parameters, viz. cost function (C), kernel gamma (γ) and width of the loss function (ε).

RESULTS AND DISCUSSION

SYNTHESIS

Syntheses of the targeted α-aminophosphonate derivatives (4a-q) were prepared through a three component reaction involving aldehydes, amines and phosphites employing Kabachnik-Fields reaction[15] condition (Figure 1). To the best of our knowledge, no previous reports using this catalyst for such reaction have been reported. All the synthesized compounds have been evaluated for their c-Src kinase inhibitory activity. The IC_{50} value (μM) was determined for each compound. Staurosporine and PP2 were employed as the positive controls.

Table 1: Molecular Docking Analysis of Phosphorylated and Unphosphorylated c-Src Kinase Tyrosine Protein with Selected Compounds. The Binding Energies were Calculated using Glide v6.8 Docking Tool. No Docking Score was Obtained

Sr. No.	Compound	Unphosphorylated Protein: 1Y57		Phosphorylated Protein: 2H8H	
		Amino acids involved in intermolecular interactions	GLIDE Score (kcal/mol)	Amino acids involved in intermolecular interactions	GLIDE Score (kcal/mol)
1	4c	Lys295 Asp404	-4.5	Lys295 Ala390	-5.0
2	4i	Asp404	-4.4	Lys295	-4.6
3	4j	Lys295 Ala390	**-5.4**	Asn391 Lys295	**-6.5**
4	4p	-	-	Ala390 Ser345	-6.10
5	4m	-	-	Lys295 Ala390	-4.18
6	Staurosporine	Met341	-8.3	-	-
7	PP2	Met341	-7.6	-	-

Out of the seventeen compounds screened, three compounds (4c, 4j, 4o) showed moderate Src kinase inhibition in the range of IC_{50} values between 15.8-63.6 μM. In particular, compound 4j, was identified as the potent inhibitor (IC_{50}=15.8 μM) in the series.

MOLECULAR DOCKING ANALYSIS

With an objective to explore the binding potential of the synthesized and tested molecules for Src tyrosine kinase, we performed docking studies as shown in Table 1. Among the 17 structures, compounds 4c, 4i and 4j were mainly investigated further for docking simulations since they exhibit higher inhibitory activity as ascertained from their MIC data. Human c-Src tyrosine kinase is a multi-domain protein consisting of 535 amino acids possessing SH3, SH2 domains followed by a short N-terminal and C-terminal regulation segment[16]. In the unphosphorylated structure, the SH2 and SH3 domains lie at a right angle to each other, with only SH3 domain in contact with the N-terminal lobe[17]. The SH3 domain is bound between SH2 and kinase domains in this inactive conformation[18]. The C-terminal tail folds back to N-terminal, and the active site is exposed as it is not blocked by the activation loop[19]. Upon phosphorylation of Tyr527 in C-terminal or Tyr 416 in the activation loop, the SH2 and SH3 domains lie parallel to the N and C lobes[20] forming an open and closed cleft that determines the access to the catalytic site[21]. Phosphorylation of Tyr 527 down regulates the kinase activity and phosphorylation of Tyr 416 is deemed necessary for exhibiting full kinase activity. The binding site in c-Src protein is an ATP pocket present in the N-lobe region[22]. The residues lining the binding site of the protein were extracted from the X-ray data[23]. Compound 4j and 4c were bound to the Lys295 residue present in the C-terminal region of the

pocket that acts as an important site for catalysis, binding of a compound to the terminal inhibits further kinase activity. Docking against the un-phosphorylated protein (1Y57) showed that 4j performed better with -5.4 kcal/mol binding energy as compared to 4c and 4i which displayed -4.5 and -4.4 kcal/mol binding energy respectively and formed only a single hydrogen acceptor bond with Asp404 (Table 1). It is to be noted here that the compound 4j displayed a similar good docking score of -6.5 kcal/mol with the phosphorylated protein (2H8H) as well. All the synthesized compounds were docked into the ATP binding site which is located at a cleft between the N and C-terminal lobes, flanked by the hinge region, P-loop, helix α C and the activation loop. The docked orientations of the 4j compound with respect to the native ligands imatinib derivative in unphosphorylated protein (1Y57) and anilino quinazoline derivative with phosphorylated protein (2H8H) in the pocket site are depicted in Figures 2 respectively.

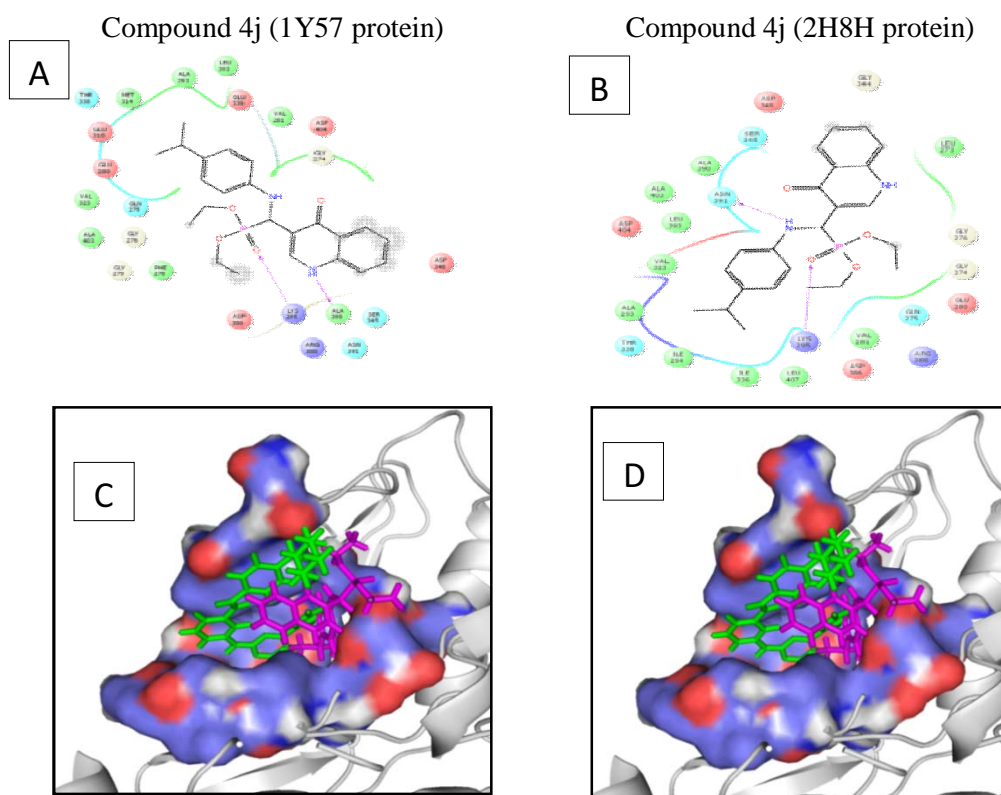Compound 4j (1Y57 protein)   Compound 4j (2H8H protein)

Fig. 2: Amino Acids Involved in Key Intermolecular Interactions for A) Compound 4j in Unphosphorylated Protein (1Y57) B) Compound 4j in Phosphorylated Protein (2H8H). C) and D) Surface View of Compound 4j in 1Y57 and 2H8H
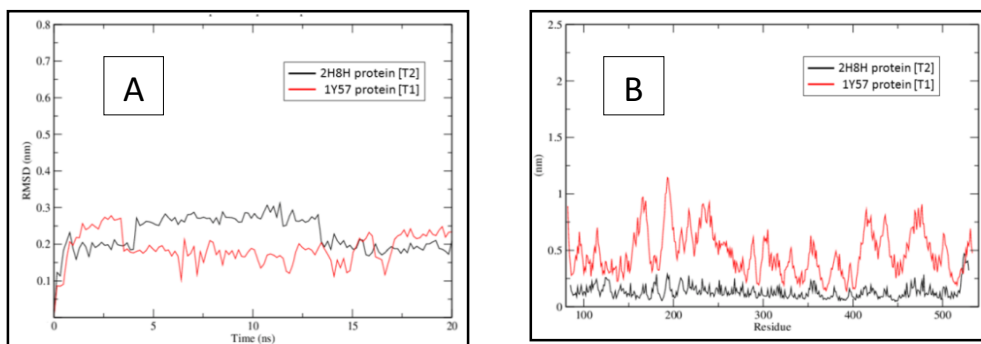
Fig. 3: A) Root Mean Square Deviation of Compound Trajectory for 2H8H (T2) and 1Y57 (T1) Protein. The RMSD Value Indicates Stability of Compound Molecules in the Pocket Region. B) Root Mean Square Fluctuation (RMSF) of Protein-compound Complex for 2H8H (T2) and 1Y57 (T1) Protein

DOCKING STUDIES OF 4P AND 4M WITH PHOSPHORYLATED PROTEIN

It is to be noted that the isopropyl aniline and methyl groups of compound 4j and 4p respectively fit in the pocket regions, whereas the isopropyl aniline group of compound 4m was observed to protrude out of the pocket region thereby leading to a lower binding efficiency (Table 1). In the docked conformation of 4p, the methyl group did not fit well into the active site cavity. These observations indicate that the *para*-orientation of the isopropyl group in the aniline ring in compound 4j may have an important role in anchoring it within the active site of the receptor.

MOLECULAR DYNAMICS STUDY

Molecular dynamics studies were performed for further probing the dynamicity and fluctuations of ligand at the atomic level in the active sites of the unphosphorylated protein (1Y57) and phosphorylated protein (2H8H) hereafter referred to as T1 and T2 respectively. Figure 3a shows the RMSD trajectories of T1 and T2. In the case of T1, the early peak in RMSD of compound 4j during the initial time scale of simulation indicates the relaxation of the compound. It reached equilibration after 3ns, reflecting its stability in the pocket region. The RMSD trajectory for T2 was stable with a rise at 3ns, but is observed to be relaxing back at 13ns. In order to study the behaviour of the compound in a dynamic environment, the root mean square fluctuation (RMSF) of protein-compound complex was performed. The RMSF trajectory (Figure 3b) was more stable in case of T2 as compared to T1; this can be attributed to the open conformation of T1 causing high fluctuations in the loop regions. The residues in the active site of the both T1 and T2 ranged from 273-295, 338-341 and 404-406 residues. Rhythmic fluctuation of compound, the amino group of Lys295 and oxygen group of Ala390 led to increase in distance, causing

dissociation of the hydrogen bond formed during docking. Dynamics studies showed that Leu273, Gly274 and Val281 from Glycine rich and P-loop region; Lys393 from Activation loop and Ala293 from β3 of the N-terminal lobe may help in understanding the residues playing crucial role in ligand binding. Targeting the N-terminal lobe can inhibit the regulatory mechanism in Src kinases. Inhibiting Leu393 present in the the regulatory activation loop, may inactivate tyrosine phosphorylation in Src and other kinases. The docking also may also affect nucleotide binding and phosphotransfer that occur in the cleft between the two lobes. Interaction of chromone derivatives to the glycine rich G loop region/P loop region, may affect the bound nucleotides. The adenine moiety of the bound nucleotide is coordinated largely by interactions with the N lobe and a short hinge segment that connects the two lobes. Dynamic studies reveal that inhibion studies varies between phosphorylated and unphosphorylated proteins due to certain conformational changes in the active site which render (i) displacement of the C helix, which removes the catalytically important residue from the active site cleft, (ii) change in the activation loop adopting an α-helical conformation, which precludes binding of peptide substrates and also sequesters Tyrosine residue, making it inaccessible for phosphorylation, and (iii) constrain in the orientation of the N and C lobes which may not be optimal for catalysis. Thus carrying out a molecular modelling studies against phosphorylated and unphosphorylated cSrc kinase protein using a synthesized chromone derivatives will help us to develop a better cSrc kinase inhibitor.

STRUCTURE ACTIVITY CORRELATION MODEL

We extracted IC_{50} data from literature for the reported Src kinase inhibitors and computed 2D molecular descriptors and principal components[24]. The 2D PCA plot displays the chemical space occupied by the newly synthesized compounds with respect to the known Src inhibitor space. A CC of 0.835 was obtained when the 17 synthesized compounds were applied to the model (Table 3). Supplementary Table 3 shows the predicted IC_{50} values when applied to the SVR model. The IC_{50} of compound 4j predicted was similar to the value predicted by experimental methods. The clues obtained from the structure correlations studies and help in fine tuning the presently synthesized compounds for further enhancing the bioactivity.

Table 2: Statistical Results Obtained for Known Src Kinase Inhibitors using SVR Model (R^2 = 0.914)

Data Set	SVR Analysis		
	CC	MAPE	RMSE
Total	0.956655	0.176301	4.087586
Training set	0.945926	0.238429	4.333623
Test set	0.983458	0.06091	4.450468
Validation set	0.999823	0.002656	0.001257

Table 3: Statistical Results Obtained for 17 Compounds using SVR Model (R^2= 0.835)

Data Set	CC	SVR Analysis	
		MAPE	RMSE
Total	0.835001	0.36019	50.99193
Training set	0.956363	0.371753	4.141958
Test set	0.756247	0.451966	84.40276
Validation set	0.833723	0.557296	95.83819

CHEMOINFORMATICS ANALYSIS

A druggability check was performed for all the 17 synthesized compounds (Supplementary Table 4). Lipinski rule of 5 predictions were performed using the Screening Assistant 2 tool[25]. The notion that these compounds could be further developed as anti-cancer compounds was further assisted by ADME properties predicted using PreADMET software[26]. The selected compound **4j** prioritized in this study fulfilled the above criteria indicating that it may be further developed in an oral dosage form[27]. TPSA (Topological polar surface area) results indicated satisfactory values for all the 17 compounds. Thus most synthesized compounds predicted favorable ADME predictions. LAZAR (Lazy structure activity relationships) software detects carcinogenic properties based on the similarities in functional group with carcinogenic compounds present in the Lazar database[28].

CONCLUSION

In summary, a series of chromone/ azachromone fused α-aminophosphonate conjugates have been synthesized using silica chloride as a new catalyst. All the synthesized compounds were evaluated for their c-Src kinase inhibitory activity and one of the compounds 4j was found to be effective with an IC$_{50}$ value of 15.8 μM. The stability and convergence of dynamics results for the phosphorylated and unphosphorylated proteins with 4j compound in the simulations establishes its potential as an efficient inhibitor. Thus Docking and dynamics studies revealed the compound to be more effective against the phophorylated form of Src kinase. Results also showed that Leu273, Gly274 and Val281 from Glycine rich and P-loop region; Lys393 from Activation loop and Ala293 from β3 of the N-terminal lobe could be considered as potential binding sites for cSrc kinase proteins.

ACKNOWLEDGEMENTS

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Anticancerous Effect of *Piper Longum in vivo* and *in Vitro* Explants on Leukemic Cell Line K562

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ABSTRACT—*Piper longum is a medicinal plant processing anticancerous activity. It's anticancerous effect was checked on leukemic cell line K562. K562 cells, were the first human immortalized myelogenous leukemia cell line. K562 cells are of erythroleukemia type, the line is derived from a 53 year old female who was chronic myelogenous leukemia patient. MTT (3-(4,5- dimethylthiazol–2–yl)-2,5-diphenyl tetrazolium bromide) assay was performed and percentage viability was estimated by using PBS(Phosphate Buffer Saline) as positive control.MTT assay depends on the capability of mitochondrial dehydrogenase enzyme from live cells to cleave the tetrazolium rings of yellow colour MTT, which results in the formation of dark blue formazan crystal, the coloured formazan products is directly proportional to the number of cells which survived.Further percentage cytotoxicity was calculated by the respective formula. Hot and cold extracts were prepared for both leaf and stem explants.Leaf and stem explants of the plant yield different result for both in vivo and in vitro study, moreover in vivo results were better than in vitro results and leaf explant results were better than that of stem explant result.*
KEYWORDS: *Piper Longum, Leukemic Cell Line K562, MTT, PBS*

INTRODUCTION

Common name of *Piper longum* is pippali, Indian long pepper and pipal [1]. *Piper longum* is a medicinal plant having anticancerous properties, hot and cold extracts of explants of *Piper longum* were prepared for *in vivo* and *in vitro* studies [2, 3, 4]. Knowledge about explants of *Piper longum* i. e, leaves and stems *in vivo* and *in vitro* anticancerous activity provides information of better medicinal power of explants of *Piper longum*. Anticancerous activity was previously determined in fruits of *Piper longum,* byestimating its cytotoxicity on cancerous cell line. [5] in present study anticancerous activity is determined in leaf and stem explants of *Piper longum*. Results of above mentioned activity was much better in case of fruits, leaf and stem explants yield minor result. The percentage cytotoxicity was low in case of later. K562 cells are of erythroleukemia type i. e blood cancer cell line, the line is derived from a 53 year old female chronic myelogenous leukemia patient. The anticancerous effect of different explants on K562 cell line is beneficial in yielding information for treatment of many human diseases and ailments resulting from blood cancer in future.

MATERIALS AND METHODS

Piper longum plant was procured from botanical garden of National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Pune, Maharashtra, India. Cancerous cell line K562 was procured from National Centre for Cell Science (NCCS), Pune, Maharashtra, India. Chemicals and reagents used were, ethyl acetate, hexane, 3-4,5-Dimethylthiazol-2yl-2,5-Diphenyl Tetrazolium Bromide (MTT), and Phosphate buffer saline (PBS)„Distilled water(DW), Indole acetic acid(IAA), Benzyl amino purine (BAP),Kinetin(KIN).

PREPARATION OF HOT EXTRACTS

10 gm powder of leaf /stem explants of Piper longum was taken and mixed with 100 ml ethyl acetate it was kept overnight on incubator shaker, next day driying was done in rotavapour, then what man filter paper was used for filtering and further was preserved at 4 C

PREPARATION OF COLD EXTRACTS

10 gm powder of leaf /stem explants of *Piper longum* was taken and mixed with 100ml hexane: water (1:1) and kept overnight on incubator shaker, next day driying was done in rotavapour then whatman filter paper was used for filtering and further was preserved at 4 C.

Determination of Anticancerous Activity of *Piper Longum in Vivo and in Vitro*

3-4, 5-Dimethylthiazol-2yl-2, 5-Diphenyl Tetrazolium Bromide (MTT) assay was performed to determine cytotoxicity, This assay was performed in ELISA reader plate.

At first percentage viability was estimated by using PBS as positive control further percentage cytotoxicity of plant's explants were checked on leukemic cell line K562 (cancerous cell line), so that its anticancerous effects can be studied. Explants of *Piper longum* taken from outside environment are used for *in vivo* analysis whereas explants which were inoculated in test tubes and phytajar under aseptic condition by the process of tissue culture results in formation of callus, which was used for *in vitro* analysis, thus the extent of anticancerous activity of different concentrations of *in vivo* and *in vitro* leaf and stem explants of *Piper longum* was determined.

FOR *IN VIVO* STUDY

$200\mu l$ of sterile Phosphate Buffer Saline (PBS) was added in 9 wells each in 1st row of wells of microtiter plate, 100 μl of cell suspension and100 μl of hot ethyl acetate extract of plant (hot extrac) t was added in 2nd row of wells. It was done in triplicate

$200\mu g/ml$, $400\mu g/ml$ and $800\mu g/ml$ of plant (hot extract) was added in 3 wells each, $100~\mu l$ of cell suspension and $100~\mu l$ of cold hexane: water extract (cold extract) of plant was added in the 3rd row of wells. It was done in triplicate $200\mu g/ml$, $400\mu g/ml$ and $800~\mu g/ml$ concentration of plant cold extract was added in 3 wells each, $200~\mu l$ of cell suspension was added in 9 wells each in the 4th row of wells it was taken as negative control. After pipetting the plate incubation was done at 37 C at 5% CO_2 for 24hrs and then 48hrs and 72 hrs. Further100 μl MTT solution was added to each well. Incubation of plate was done in dark at 37 C in CO_2 incubator for 4hrs.Absorbance was taken at 570 nm in ELISA reader and OD obtained was used in formula to caculate cell viability and cell cytotoxicity. The *in vivo* and *in vitro* percentage in each case was calculated by taking mean in triplicate of sample's optical density and the results were obtained from respective formulae standard deviation is shown in respective graphs in fig1 and fig. 2. [5]

FOR *IN VITRO* STUDY

Sterilization of explants (leaves and stem of *Piper longum)* was done and inoculated in M.S. hormonal media using IAA, BAP and Kinetin as growth regulators for callus induction. Callus was used for *in vitro* study. *In vitro* grown callus were cut into pieces and crushed with the help of motor and pestle with distilled water and then 10 ml of solution was added to conical flask containing 90 ml of distilled water.

The steps for preparation of hot and cold extracts were same as *in vivo*.

% viability= (OD of treated cells/OD of control cells) x 100

% of Cytotoxicity= 100 – [(O D of the treated cells/O D of the control cells) x 100]

RESULT AND DISCUSSION

In vivo results were better than *in vitro* results and hot extract were better than cold extracts for both leaf and stem of *Piper longum*. In this case PBS was taken as positive control. For Hot extract and cold extact *in vivo* and *in vitro* leaf and stem explants– Anticancerous % cytotoxicity = 100 – [(OD of treated cells/OD of control cells)x100].In case of hot extract of (leaf) and (stem) *in vivo* and *in vitro,* for 200 $\mu g/ml$ of plant sample % cytotoxicity was 3.68% and 2.18%in case of *in vivo* for leaf and stem respectively and2.84% and2.04% in case of *in vitro* for leaf and stem respectively, for 400 $\mu g/ml$ of plant sample % cytotoxicity was 4.58% and 3.25%in case of *in vivo* for leaf and stem respectively and 4.40% and3.25% in case of *in vitro* for leaf and stem respectively, for 800 $\mu g/ml$ % cytotoxicity was 5.03% and3.84% in case of *in vivo* for leaf and stem respectively and 4.91% and 3.45%in case of *in vitro* for leaf and stem respectively,% of positive control was 7.78% and 6.28% in case of *in vivo* for leaf and stem respectively and 8.36% and 7.19% in case of *in vitro* for leaf and

stem respectively, in case of cold extract of (leaf) and stem *in vivo* and *in vitro*, for 200 μg/ml of plant sample % cytotoxicity was 2.28% and 1.80% in case of *in vivo* for leaf and stem respectively and 2.13% and 1.74%in case of *in vitro* for leaf and stem respectively, for 400 μg/ml of plant sample % cytotoxicity was 3.63%and3.12%% in case of *in vivo* for leaf and stem respectively and 3.24 %and 2.98% in case of *in vitro* for leaf and stem respectively, for 800 μg/ml % cytotoxicity was 3.94% and3.29% in case of *in vivo* for leaf and stem respectively and 3.65% and3.16% in case of *in vitro* for leaf and stem respectively,% of positive control was 7.78% and 6.28% in case of *in vivo* and 8.36% and 7.19% in case of *in vitro* for leaf and stem respectively.

DISCUSSION

In previous study carried out on *Piper longum* fruits to check its anticancerous activity showed dose dependent increase in % cytotoxicity (5,6,7,8) in present study carried on *Piper longum* leaf and stem explants also showed dose dependent increase in % cytotoxicity but low % was obtained in each case as compared to fruit extract. Thus the above mentioned protocol can further be used to determine % cytotoxicity of different medicinal plant on other cancerous cell line which will be beneficial in treatment of diseases related to leukemia. In present study knowledge was obtained by knowing the extent of cytotixicity on leukemic cell line K562.The comparative graph plotted for *in vivo* and *in vitro* result in different concentration and respective standard deviation are shown in fig1 and fig 2 respectively.
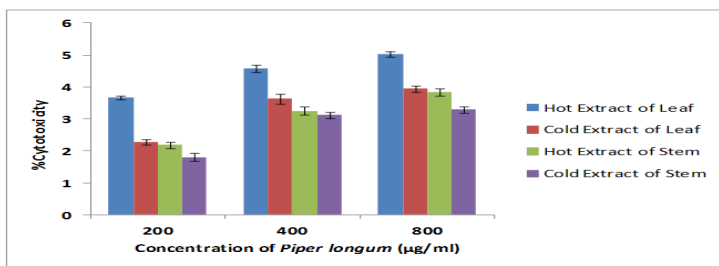

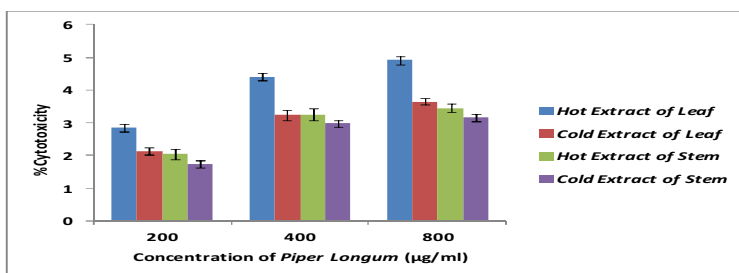**Fig. 1: Comparison of *in Vivo* Results of Anticancerous Activity of *Piper Longum***

Fig. 2: Comparison of in Vitro Results of Anticancerous Activity of Piper Longum

CONCLUSION

It was concluded by the findings that knowledge acquired by the results of anticancerous activity of plant are of great importance to mankind, as by determining its anticancerous, activity many diseases can be treated, information about *in vivo* and *in vitro* explants (leaf and stem) of *Piper longum* for hot and cold extracts contributed to plant's medicinal uses in case of antitumor studies, further the difference in, *in vivo* and *in vitro* results, shows different pecentage cytotoxicity in each case moreover anticancerous effect on leukemic cell line K562 was observed. Low percentage cytotoxicity was obtained in present study thus minor results were obtained, further above protocol can be used to study anticancerous activity of different medicinal plant on some other cancerous cell line, in future.

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COMPETING INTERESTS

No competing interests exist.

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Computational Modelling of *Tetradon nigrovirdis* Melanocortin-1 Receptor (MC1R) Gene and Identification of Natural Compounds as Putative Modulator

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ABSTRACT—*Body ornamentation in fishes is of great economic importance. A highly polymorphic Melanocortin-1 receptor (MC1R) gene is primarily responsible for pigmentation in fishes since it acts as an initiator gene for the melanogenesis pathway. The green spotted puffer fish Tetradon nigrovirdis, with the green colored body and black spots is a favorite aquarium fish. T. nigrovirdis is widely used as a model organism in genetic studies and is also a useful candidate species to understand the color inheritance in fish species. Modulation of MC1R protein will help to understand the body color formation in fish species. Considering these aspects the present study was aimed to predict the tertiary structure of the MC1R protein of T. nigrovirdis and to identify putative small molecules as the modulator of MC1R. The present study identified that the MC1R gene of T. nigrovirdis encodes for a protein consisting of 325 amino acids. The conserved domain information revealed that MC1R gene belongs to the G-protein coupled receptor (GPCR) family. The molecular weight of the MC1R protein was 37043.99, and its theoretical pI was 6.61, indicating it as an acidic protein. MC1R protein is composed of seven transmembranes, localized in the plasma membrane and the active sites are mainly present in the TM 5 (200-222 amino acid residue) and TM 6 (243–265 amino acid residue) region. Since the tertiary structure of the MC1R protein of T. nigrovirdis is not available, the same was predicted by homology modeling using the turkey beta1 adrenergic receptor, chain A (PDB ID - 4IAR.1.A) protein as a template, with a sequence identity of 30.94%. The analysis of the tertiary structure by the Ramachandran plot revealed that the 91.1% of the residues were within the most favored region indicating the adequacy of the predicted model. A total of 1,80,313 natural compounds, extracted from ZINC database were virtually screened for their ability to bind the identified transmembrane region of MC1R. The analysis of the results for ADME properties showed that the Blahtetrone, extracted from camu berry, had the lowest binding efficiency (-9.9 kcal/mol), and was identified as a putative modulator of the MC1R protein.*

KEYWORDS: *Melanocortin 1 Receptor (MC1R) Gene, Tetradon Nigrovirdis, Homology Modeling, Virtual Screening, Blahtetrone*

INTRODUCTION

Body ornamentation in fishes is of great economic importance. A highly polymorphic Melanocortin-1 receptor (MC1R) gene initiates the melanogenesis pathway and is primarily responsible for pigmentation in fishes. The green spotted puffer fish *Tetradon nigrovirdis*, with the green colored body and black spots is a favorite aquarium fish and is a model organism in genetic studies including the color inheritance.

MC1R is a part of the melanocortin (MC) receptor family, which is the smallest member of class A (rhodopsin-like) family of G-protein coupled receptors (GPCRs) (Horrell *et al*., 2016). Melanocortin family comprises of five members: MC1R, MC2R, MC3R, MC4R and MC5R possessing different functions and tissue profiling. MC1R is mainly responsible for coloration and UV-resistance and is abundant in both leukocytes and melanocytes. In fishes, MC1R signaling mainly contributes to the amount and type of melanin pigments synthesized by melanocytes, regulating both basal pigmentation and the UV induced tanning response (D'Orazio *et al*., 2006). The active form of MC1R in the presence of α-MSH produces eumelanin, which produces dark coloration (Virador *et al*., 2002). The inactive form of MC1R produces the pheomelanin which provides red or yellow color.

The reviewed literature suggests that the MC1R is present in almost all fish species and has an active role in skin pigmentation. Skin color determination in fish is a complex process involving a series of genetic, cellular and physiological factors which together determine the external appearance of a fish (Colihueque, 2010). Body coloration is an important economic trait of ornamental fishes and understanding the phenomena color formation will help to develop an improved strain. Considering the vital role of the MC1R gene in color formation, in the present study, we computationally characterized the MC1R gene of *T. nigrovirdis* and identified the natural compounds as putative modulators of MC1R protein.

MATERIALS AND METHODS

DATA COLLECTION

The protein sequence of melanocortin receptor gene of *Tetradon nigrovirdis* was retrieved in FASTA format, with the help of UniProt. The UniProtKB ID for *T. nigrovirdis* was H3CV70 (Jaillon, 2004 and Yun, 2015).

SEQUENCE ANALYSIS

BLAST CD-search (https://www.ncbi.nlm.nih.gov/Structure/cddi) was used to identify the conserved domain regions of the protein sequence.

PRIMARY STRUCTURE ANALYSIS

The physical and chemical properties of the MC1R protein were determined using ProtParam tool (https://web.expasy.org/cgi-bin/protparam/protparam). The transmembrane information was determined by applying the hidden Markov model using the TMHMM v. 2.0 (Trans-membrane Hidden Markov Model) server (http://www.cbs.dtu.dk/services/TMHMM/). Hum-mPLoc v3.0 was used to predict the subcellular localization of the protein (Hang, 2016), (http://www.csbio.sjtu.edu.cn/bioinf/Hum-mPLoc3/).

SECONDARY STRUCTURE ANALYSIS

For the prediction of positional possibilities of α helix, β sheets, loops and coils, the SOPMA server (Geourjon and Deleage, 1995) was used.

TERTIARY STRUCTURE PREDICTION

Tertiary structure for the MC1R amino acid sequence was predicted by employing homology modeling with the help of SWISS-MODEL (https://swissmodel.expasy.org/) (Biasini *et al.*, 2014). The best model was selected based on the Global Model Quality Estimation (GMQE) parameters. The predicted model was validated by Ramachandran plot generated with the help of PDBsum server (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html). Python Molecular Viewer (PyMol) was used to visualize the tertiary structure of the protein (Sanner, 1999).

ACTIVE SITE PREDICTION

COACH server (https://zhanglab.ccmb.med.umich.edu/COACH/) (Yang *et al.*, 2013) was used for predicting the active site residue. The result was based on template selection, which further depends upon the confident score (C-score), which lies in the range of 0-1, between the query and the template.

NATURAL COMPOUNDS LIBRARY PREPARATION

Natural compounds library was prepared to study the docking properties with the protein structure. These special subsets were downloaded from the ZINC12 database (www.zinc.docking.org). A total of 180313 natural compounds were downloaded in the SDF format. The compounds were made docking ready into the PDB format followed by PDBQT with the help of OpenBabel software. The zip file was split into single files with the help of vina split software.

PROTEIN-LIGAND DOCKING

PDB format of the protein files was converted to PDBQT format by the help AutoDock tool. Hydrogen ions and charges were added to form a stable PDBQT structure. Grid box, for docking, was made with the help of AutoDock tool. Different types of docking were performed based on the complete structure docking and at different active site positions.

LIGAND PREDICTION

The criterion for selecting the ligands was based on their lowest binding efficiency, threshold value being -7 kcal/mol, and their ADME properties, which was tested by using ADMETsar server (http://lmmd.ecust.edu.cn/admetsar1/predict/)

RESULTS AND DISCUSSION

SEQUENCE ANALYSIS

The MC1R gene of *T. nigrovirdis* had 978 bp and it encodes 325 amino acids. The conserved domains information revealed that MC1R genes belong to the G-protein coupled receptor (GPCR) family. The MC1R protein is having three domains, which lie in the range of 62–302 amino acid sequence. The first domain, found at the N-terminal, was the chemoreceptor and is termed as a serpentine type 7TM GPCR Srsx domain. The second domain was from the class A-Rhodopsin family, which is a seven transmembrane receptor. The third domain was present at the C-terminal and is known as the G-protein coupled chemokine receptor. Presence of chemoreceptor in the domain depicts that MC1R protein is capable of taking other compounds into it.

PRIMARY STRUCTURE ANALYSIS

The molecular weight of H3CV70 was predicted to be 37043.99 Dalton and the theoretical pI 6.61, indicating a negatively charged, acidic protein. The subcellular localization prediction showed that the MC1R is a transmembrane protein and is present in the plasma membrane. The analysis using TMHMM server showed that the H3CV70 MC1R protein was composed of seven trans-membranes and the sequence position are provided in Table 1, and the posterior probability of the transmembrane is shown in Fig. 1.

Table 1: Expression of MC1R Protein Amino Acid Residues in Different Regions

| Query Accession number	Server	Transmembrane region information	Starting Amino acid	End Amino acid
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	outside	1	50
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	51	70
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	inside	71	82
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	83	105
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	outside	106	124
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	125	147
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	inside	148	167
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	168	190
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	outside	191	199
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	200	222
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	inside	223	242
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	243	265
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	outside	266	279
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	TMhelix	280	302
tr|H3CV70|H3CV70_TETNG	TMHMM2.0	inside	303	325
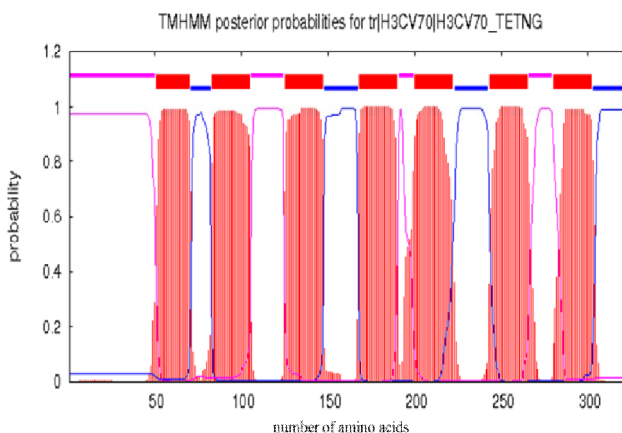
Fig. 1: Transmembrane Topology Prediction of MC1R Protein by using TMHMM Server. Red Lines Depict the Transmembrane Region of the Protein While Blue and Pink Line Represents the Inside and Outside Membrane Region Respectively

SECONDARY STRUCTURE ANALYSIS

Analysis of the predicted secondary structure of the MC1R protein showed that it is composed of 44.00% Alpha helix, 27.38% strands, 7.08% Beta-turns and 21.54% coils (Table 2). The presence of a high number of α-helices indicates that the MC1R protein is highly stable and robust to mutations (Abrusán and Marsh, 2016).

Table 2: Secondary Structure Prediction of *T. nigrovirdis* using SOPMA Server

Protein Structure Unit	No. of Amino Acids	Percentage of Structural Unit
Alpha helix	143	44.00
3_{10} helix	0	0.00
Pi helix	0	0.00
Beta bridge	0	0.00
Extended strand	89	27.38
Beta-turn	23	7.08
Bend region	0	0.00
Random coil	70	21.54
Ambiguous state	0	0.00
Other states	0	0.00

TERTIARY STRUCTURE ANALYSIS AND VALIDATION

Chain-A of beta1 adrenergic receptor protein of turkey was used as a template for the tertiary structure prediction as it had 30.94% amino acid sequence identity with *T. nigrovirdis* MC1R protein. The predicted tertiary structure is shown in Fig. 2. The analysis of the tertiary structure showed the GMQE of 0.52 and QMEAN of -3.69 between the query sequence and the template. The analysis of Ramachandran plot

[60]

revealed that of the 325 amino acid residues 234 (91.1%) were found in the most favored region and the remaining 23 (8.9%) amino acid residues in the additionally allowed regions (Fig. 3), indicating the high crystallographic quality of the predicted tertiary structure. The analysis further confirmed the presence of seven transmembrane regions in the protein predicted by using TMHMM server.
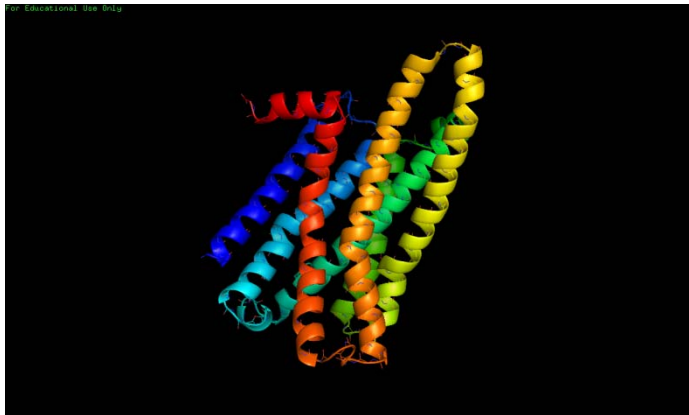

Fig. 2: Predicted Tertiary Model of MC1R Protein of *T. nigrovirdis* by using SWISS-MODEL, based on Homology Modelling, Viewed in PyMol
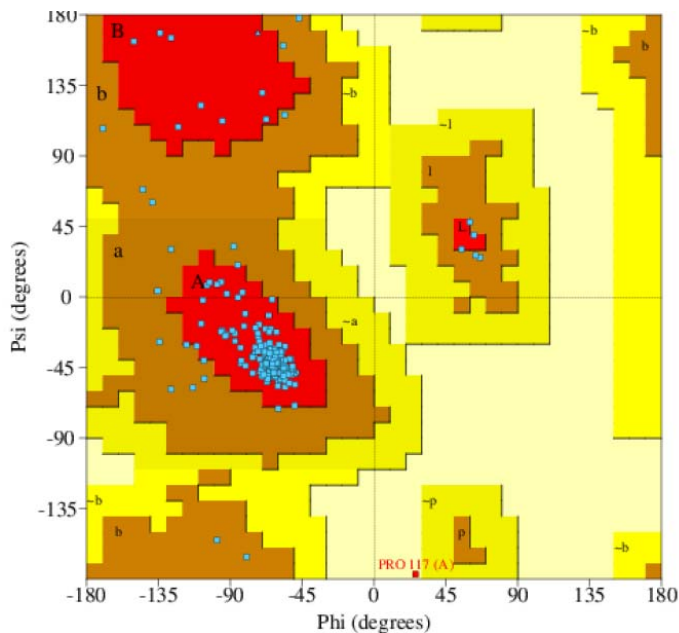

Fig. 3: Ramachandran Plot of the Predicted Tertiary Structure of MC1R Protein of *T. nigrovirdis* by PDBsum Server

ACTIVE SITE PREDICTION

C-score for the H3CV70 was found to be 0.34 with the template of 2ycxA_BS01_P32. The active site residues predicted, using COACH server, were I-127, D-128, I-131, C-132, L-199, A-201, T-206, F-209, W-258, F-261, F-262, L-265, L-288, I-292, indicating that the active site residues are found in TM-5 (200-222 amino acid residues) and TM-6 (243-265amino acid residue).

DOCKING AND LIGAND PREDICTION

Based on the minimum binding efficiencies of the ligands with the protein, three ligands from 180313 were selected which are presented in Table 3. The estimated ADME properties proved that the selected compounds were suitable for the usage in fishes. The natural compound Blahtetrone, extracted from *Camu camu* berry, a widely found berry in Africa (ZINC ID - ZINC05220992) was found to have potential to be a putative modulator of the MC1R protein of *T. nigrovirdis*. Blahtetrone's use as a chemical has not been reported to date.

Table 3: Binding Efficiencies of the Natural Compounds to the MC1R Protein of *T. nigrovirdis*

Zinc ID	Binding Efficiency (kcal/mol)
ZINC05220992	-9.90
ZINC20763420	-9.70
ZINC04237106	-8.30

CONCLUSION

The predicted tertiary structure of *T. nigrovirdis* MC1R protein in this study provides an insight in to the MC1R structure and function. The MC1R protein of *T. nigrovirdis* is highly stable and robust to mutations. The identified modulators may help to alter the coloration in *T. nigrovirdis*. This study allows to understand and explore the activities of MC1R protein of other fish species and helps to develop novel modulators for those species.

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Prediction of Risk of Breast Cancer by Damaging SNPS of NEIL1 Glycosylase

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ABSTRACT—*Base Excision Repair pathway has pivotal role in rectification of damaged bases caused by reactive oxygen species. NEIL1 is one of the important glycosylases which removes the damaged base by breaking the glycosidic bond between deoxyribose sugar and base. These damaged bases needs to be corrected to maintain genomic integrity. Single nucleotide polymorphisms are reported to be deleterious and predisposing to several diseases like cancer and aging. In our study, we analysed the association of three SNPs (rs5745906 (G83D), rs3734091 and rs6869366) with breast cancer in the population of West India. SNP rs7182283 and rs4462560 were found to be significantly associated with breast cancer.*

KEYWORDS: *Deleterious, DNA Repair, Non-Synonymous, Polymorphism*

ABBREVIATIONS

ARMS	=	Amplification-refractory mutation system
BER	=	base excision repair
CI	=	confidence interval
DDG	=	Gibbs free energy change
HMM	=	Hidden Markov Model
HWE	=	Hardy–Weinberg equilibrium
NCBI	=	National Center for Biological Information
NEIL 1	=	Nei End onuclease VIII-Like 1
ns SNPs	=	non-synonymous single nucleotide polymorphisms
OR	=	odds ratio

PANTHER = Protein ANalysis THROUGH Evolutionary Relationships

PDB = Protein Data Bank

RI = reliability index

ROS = Reactive oxygen species

SIFT = sorting intolerant from tolerant

SNPs = single nucleotide polymorphisms

sub PSEC = -substitution position-specific evolutionary conservation

SVM = support vector machine

INTRODUCTION

Reactive oxygen species (ROS) are the major cause of DNA damages in mammalian cells [1]. Single base changes due to ROS include 8-hydroxyguanine, 2, 6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyadenine etc. [2]. Base Excision Repair (BER) pathway has pivotal role in rectification of damaged bases caused by ROS. These damaged bases needs to be corrected to maintain genomic integrity which otherwise leads to development of diseases like cancer and aging. DNA glycosylases are the first enzymes of BER pathway that removes these damaged bases. NEIL1 protein which is one of the major glycosylases removes the damaged base by breaking the glycosidic bond between base and deoxyribose sugar [3]. The damaged bases revoed by NEIL1 includes, thymine glycol, 5-hydroxyuracil, formamidopyrimidine-G, urea, and for mamidopyrimidine-A, which are known to cause mutagenesis and cell death [4, 5]. Recent reports demonstrated somatic inactivating NEIL1 mutations and reduced NEIL1 expression in a subset of gastric cancers, suggesting that reduced NEIL1 activity is involved in gastric carcinogenesis [6]. Many studies prove that sequence variants and single nucleotide polymorphisms (SNPs) in DNA repair genes influence DNA repair capacity and make individuals susceptible to various diseases including cancer [7-9]. Several genomic variants of the human NEIL1 enzyme have been identified in some cancers during the last decade [10-13]. It is reported that heterozygote and NEIL1 knockout mice developed symptoms of metabolic syndrome [14]. Thus, it is important to study the effects of these variants on enzyme activity which influence the cancer susceptibility. Computational approach is the easy way to predict the effect of variants on protein function and structure, as a large number of variants can be screened for their effect. Various tools are available that predict the deleterious

mutations on the basis of sequence homology and structural aspects [15]. No study reported the analysis of these genetic polymorphisms with breast cancer. We tried finding effect of these *NEIL1* polymorphisms using insilico approach as well as their associated risk with breast cancer. We also studied the association of other clinical parameters with these SNPs in breast cancer patients.

MATERIALS AND METHODS

STUDY SUBJECTS

This study was approved by Human Research Ethics Committee, P.S. Medical College, Karamsad, Gujarat. Blood samples from 120 breast cancer patients (females) and 130 normal healthy females were obtained from Shri Krishna Hospital, Karamsad, Gujarat. Informed consent was obtained from both patients and controls prior to blood collection. The general/ clinical characteristics of patients are mentioned in the Table 1.

GENOTYPING OF NEIL1 SNPS (RS7182283, RS4462560 AND RS5745906)

Genomic DNA was extracted from the collected blood samples using Phenol-chloroform method. PCR-RFLP method was used to analyse the genetic polymorphisms rs 7182283(g.46434077G>T) and rs4462560 (g.46438282C>G) in the NEIL1 promoter region. The primer sets used for SNP rs7182283 were: 5'-CAACCTCCTGATTAACTGGAACCACA-3' (forward) and 5'-TCACTTCAGCCCAGGAGACCAG-3' (reverse), while for SNP rs4462560 were: 5'-GTCTCTTCACTGGCTTTTGGGG-3' (forward) and 5′-TCCCAGGTATTTGGTGGGTAGG -3′ (reverse) [16]. The cycling conditions used for rs7182283 were: one cycle at 95°C for 5 min; 35 cycles of 95°C for 30 sec, 54°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 5 min. The cycling conditions used for rs4462560 were: one cycle at 95°C for 5 min; 35 cycles of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 5 min. The PCR products of rs7182283 and rs4462560 were digested with restriction enzymes NlaIII (New England Biolabs, Beverly, MA, USA) and HaeIII (New England Biolabs), respectively, in order to detect the variants. There were 119-bp and 118-bp amplified PCR products for rs7182283 and rs4462560, respectively. The NlaIII and HaeIII restriction enzymes (New England Biolabs) were used to detect rs7182283 and rs4462560 polymorphisms, respectively, which resulted in 91-bp and 28-bp fragments in the presence of T allele (rs7182283); and 96-bp and 22-bp fragments in the presence of C allele (rs4462560).

For SNP rs5745906, we used (ARMS) technique to detect the variant allele. Primers were designed using ARMS primer design server software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html) [17]. Primer sets used were: forward outer- 5'-CCTCTGTCAGCCGCAACCCTGAGGTGCC-3', reverse outer: 5'-GTACTCCTGCAAGACACAGGGCCCGCGG-3', forward inner 5'-CCCTGGTCTTCCGCTTCGGCATGTCAGG-3' (for G allele) and reverse inner 5'-CTCGCGGGGCACCAGCTGAAAAGCGT-3' (for A allele). To confirm the results of ARMS method, we designed new primer to introduce restriction site at the end of primer. The new primer sequence is 5'-CCCTGGTCTTCCGCTTCGGCATGTCCG-3' (forward inner). The restriction enzyme used was MspI. We also used capillary electrophoresis sequencing method to confirm the results. Genetic analyser 3500 of Applied Biosystems with POP7 polymer was used.

STATISTICAL ANALYSIS

The χ^2 test was used to calculate differences between cases and controls for the selected demographic variables, age, and serum creatinine level. The correlation of genotypes and allele frequency between cases and controls were also obtained. Odds ratio and confidence of interval was obtained using Fisher exact test. Hardy–Weinberg equilibrium (HWE) of rs7182283 and rs4462560 genotypes was tested by performing a goodness-of-fit χ^2-test.

RESULTS AND DISCUSSION

GENOTYPIC ANALYSIS

The frequency distributions of selected characteristics of the cases and controls are presented in Table 1. The cases and controls were well matched for age and sex, with a mean age of 51.58 years for the cases (± 10.84 years; range, 18–85 years) and 48.87 years for the controls (± 10.79 years; range, 30–80 years) ($P = 0.99$); there was also no difference in the distribution of age groups (≤ 50, 51–64, and ≥ 65). There were no smokers and drinkers among the cases and controls. However, there were more ER positive and invasive type patients (63% and 90%, respectively) (Table 1).

Table 1: General Characteristic of Patients

Variables	Mean Age[*] (±SD)	Menopausal Status		Smoking Status	Alcohol Status	ER Status		Tumor Side		Type	
		Pre	Post			ER +	ER -	Left	Right	Invasive	In situ
Cases	51.58 ± 10.84	44	76	Nil	Nil	63	57	57%	43%	90%	10%
Controls	48.87 ± 10.79	56	74	Nil	Nil	-	-	-	-	-	-

p-value = 0.99 when cases and controls are compared

As shown in Table 2, the frequencies of minor allele (T) of SNP rs7182283 were 50% and 22.31% for cases and controls, respectively. The allele frequencies show significant difference between the cases and controls (P = 0.001), frequency of allele T being higher in patients. The frequencies of minor allele (G) of SNP rs4462560 were 48.33% and 80.77% for cases and controls, respectively. The difference between frequencies of the cases and controls was statistically significant (P = 0.00). For SNP rs7182283, GG genotype (OR =0.042, 95% CI = 0.016-0.109) was more frequent in controls (55.38%) than in cases (5%) and was significantly associated with decreased risk of breast cancer compared with TT genotype (Table 3).

To detect SNP rs5745906, we used ARMS method. This mutant showed presence of genotype GG only (Table 3 and 4). To confirm that RFLP site was introduced in the primer and the results of RFLP showed similar results with ARMS method. To further check the accuracy of RFLP method, we sequenced two randomly selected samples each of control and patient group. All four samples had homozygous wild type condition for this SNP. This suggests that this genotype is more frequent in this population. Association of it with breast cancer cannot be drawn.

Table 2: Allele Frequencies for *NEIL1*rs7182283, rs4462560, and rs5745906 Polymorphisms in the Breast Cancer and Control Groups

SNP Neil1	Genotype	Allele Frequency		Control	
		Controls (130)	Cases (120)	Chi-Square Value	p-Value
rs7182283	G	77.69%	50%	10.72	0.001
	T	22.31%	50%		
rs4462560	C	19.23%	51.67%	16.49	0.000
	G	80.77%	48.33%		
rs5745906	G	100%	100%	-	-
	A	0	0		

Table 3: Association of *NEIL1*rs7182283, rs4462560, and rs5745906 Polymorphisms and Breast Cancer risk

Genotype (Neil1)		Controls (130)	Cases (120)	Odds Ratio	95% Confidence Interval	P-Value Fisher Exact Test	Association
rs7182283	GG	72 (55.38%)	6 (5%)	0.042	0.016-0.109	0.00	Significant
	GT	58 (44.62%)	108 (90%)	11.172	5.362-23.711	0.00	Significant
	TT	0 (0%)	6 (5%)	Inf	1.187-Inf	0.011	Significant
rs4462560	CC	12 (9.23%)	18 (15%)	1.735	0.751-4.048	0.177	0.227
	CG	26 (20%)	88 (73.33%)	11.000	5.861-20.805	0.00	Significant
	GG	92 (70.77%)	14 (11.67%)	0.055	0.026-0.112	0.00	Significant
rs5745906	GG	130 (100%)	130 (100%)	-	-	1.00	Non-Significant
	GA	0	0	-	-	-	-
	AA	0	0	-	-	-	-

In this study along with the case control difference, we also tried to correlate the genotype conditions with pre-menopausal and post-menopausal status (Table 4 and Fig. 1). We observed that in case of polymorphism rs4462560, women with CC and GG genotype are only postmenopausal while women with CG genotype were both premenopausal and postmenopausal. This may suggest that the women carrying CC and GG genotype may develop the diseases at later stage of their life. In case of polymorphism rs7182283, GG, TT and GT genotypes are equally distributed in both the groups.

Table 4: Correlation of Genotypes of SNP Rs 4462560 with Premenopausal and Postmenopausal Status

Genotypes	Pre-Menopausal	Post-Menopausal
CC	0	100%
CG	26.13%	62.5%
GG	0	100%

Fig. 1 Graphical Representation of Association of Genotypes with Menopausal Status for SNP rs4462560

We also made attempts to draw association of ER status with genotype of these variants. In case of SNP rs4462560, homozygous CC and GG genotypes were found more in females with ER-ve, PR-ve and Her2+ status. Genotype CG was found in females with both ER+ve and ER-ve status. In case of SNP rs7182283, inconsistent data was observed.

Very few studies have been reported that correlated the polymorphisms of *NEIL1* with cancer. The G83D variant cause most drastic change in glycosylase activity. It displayed a significant decreased glycosylase activity compared to wild type enzyme

and had no activity with the Tg lesion [12, 18]. So it has major role in substrate recognition as proved by another study carried out by Fors bring *et al.* [10]. They observed that the G83D mutant protein displayed no activity on ss DNA containing a single 8oxoG or DHT. This shows key role of G83 in substrate recognition. So, these variants may be of significance for carcinogenesis. SNP rs5745906 is listed in the NCBI dbSNP with a very low minor allele frequency in the East Asian population (1000 Genomes project). We tried to find out the prevalence of this SNP in West Indian population. To the best of our knowledge, this ns SNP has not been studied with breast cancer in any population. So, we chose this SNP to investigate its association with breast cancer patients in the population of West India. We also included two other SNPs rs7182283 and rs4462560, which were earlier studied with other type of cancers but not breast cancer [16, 19].

In our study, we found TT genotype of polymorphism rs7182283 to be significantly associated with increased risk of breast cancer while GG genotype of polymorphism rs4462560 to be significantly associated with decreased risk of breast cancer in this population. This study is contradicting to one that reported no significant association of these SNPs with squamous cell carcinomas of the oral cavity and oropharynx (SCCOOP) [16]. Another study that involved only cases, found SNP rs4462560 to be associated with decreased risk of acute radiation-induced esophageal toxicity (RIET) and radiation pneumonitis (RP) [19].

CONCLUSION

In our study, we found TT genotype of polymorphism rs7182283 to be significantly associated with increased risk of breast cancer while GG genotype of polymorphism rs4462560 to be significantly associated with decreased risk of breast cancer in this population. However, the sample size was small. Therefore, our findings should be validated in future population-based studies that include larger numbers of patients with breast cancer and controls, and more detailed data on environmental exposure.

Conflict of Interest: The authors have declared that no conflict of interest exists.

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BM

BIOMEDICAL (ORAL PRESENTATION)

Heart Rate Variability Time-Domain Measures in Oesophageal Cancer

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ABSTRACT

Aim: Oesophageal cancer (OC) is innervated by sympathetic and parasympathetic nerves. The performance status (PS) of such patients is overlooked. There is a strong correlation between autonomic nervous system (ANS) and Heart Rate Variability (HRV). The aim of the study is to analyze the performance status of oesophageal cancer using HRV measures.

Method: Lead II Electrocardiogram of 7 OC patients and 30 healthy controls was recorded for 5 minutes using MP45 bioamplifier (Biopac Systems Inc., USA). Tachogram was obtained using Acknowledge 4.0 (Biopac Systems Inc., USA) and RR Intervals was fed to Kubios 2.1 (Finland) to obtain HRV measures.

Results: mRR, SDNN, RMSSD, NN50 and pNN50 decreased whereas mHR increased. All the measures were lower than controls, except mHR.

Conclusion: Increased sympathetic activity and decreased parasympathetic activity was found in OC with PS.

KEYWORDS: *Autonomic nervous system, Cancer, Heart Rate Variability, Time Domain, Oesophagus*

INTRODUCTION

Cancer mortality has an increasing rate and has diagnosed to be leading cause of death [1]. Oesophageal cancer which is the eighth most known cancer worldwide with mortality rate of 5.4% (occurs sixth amongst all cancer types) is found in the food pipe which lies between the throat and the stomach. The histopathological findings squamous-cell carcinoma and adenocarcinoma. The diagnosis of the disease is performed biopsy with an endoscope[2–5]. Small tumors may be operated by surgery alone but large tumors are cured by the treatment of radiation therapy and chemotherapy [6]. The esophagus receives parasympathetic and sympathetic innervation which controls the secretion from glands, internal pore of the blood vessel, and the striated and smooth muscle activity [7].

HRV is the physiological method in which the time interval between heartbeats vary. *[8].* HRV methods are non-invasive time domain, frequency domain and nonlinear methods. These methods explain ANS dysfunction, if any. Eastern Co-operative Oncology Group (ECOG) Scale is a scale of Performance Status (PS) to evaluate the severity of the disease in cancer patients, explained in our previous study*[9–12].*

It is hypothesized that time domain measures of HRV decreased in Oesophaogeal Cancer and there exists ANS dysfunction.

METHOD AND SUBJECTS

Lead II electrocardiogram (ECG) at 24ºC in supine position with controlled breathing rate was recorded for 5 minutes using MP45 bioamplifier (Biopac Systems Inc., USA) using SS2LA recording leads and disposable gel electrodes. The signal was sampled at 200 samples per second to avoid aliasing. Kubios HRV 2.1 (University of Finland, Finland) was utilized to extract HRV measures in time domain measures from the obtained tachogram. *Diabetes, hypertension, mental illness and cardiac disorder subjects have been excluded from the study [9–12].*

RESULTS

Mean of RR intervals (mRR) as shown in *Fig. 1 (a),* Standard deviation of RR interval (SDNN) in *Fig. 1 (b),* Root Mean Square of successive differences of RR intervals (RMSSD) in *Fig. 1 (c)* decreased from ECOG3 to 4. Average of Heart Rate (mHR) in *Fig. 1 (d)* increased from ECOG3 to 4. Controls had higher values of mRR, SDNN and RMSSD and lower values of mHR than OC. All the significant findings of time domain measures of HRV is given in Figure 1.

DISCUSSION

mRR, SDNN, RMSSD decreased and mHR increased in the present study which is similar to published results where it decreased in lung cancer and pulmonary metastases. There exists increased sympathetic activity and decreased parasympathetic activity *[9–11]. Decreased RMSSD indicates decreased parasympathetic activity and sympathetic dominance[13].* Adjacent RR intervals differing by more than 50 ms (NN50) and (percentage of NN50) pNN50 have many zeroes, hence was not considered in the current study. Triangular Index (TI) and Triangular Interpolation of RR intervals (TINN) were not considered for the study because it is not significant for short term recordings *[14].*

CONCLUSION

There exists autonomic dysfunction in OC which is severe in ECOG4. *Clinicians can give the treatment as per the known severity of the disease indicated by PS. HRV measures can help clinicians to understand the PS of the patients which can be an added tool to confirm the severity of the disease, thereby improve the quality of their patients.*
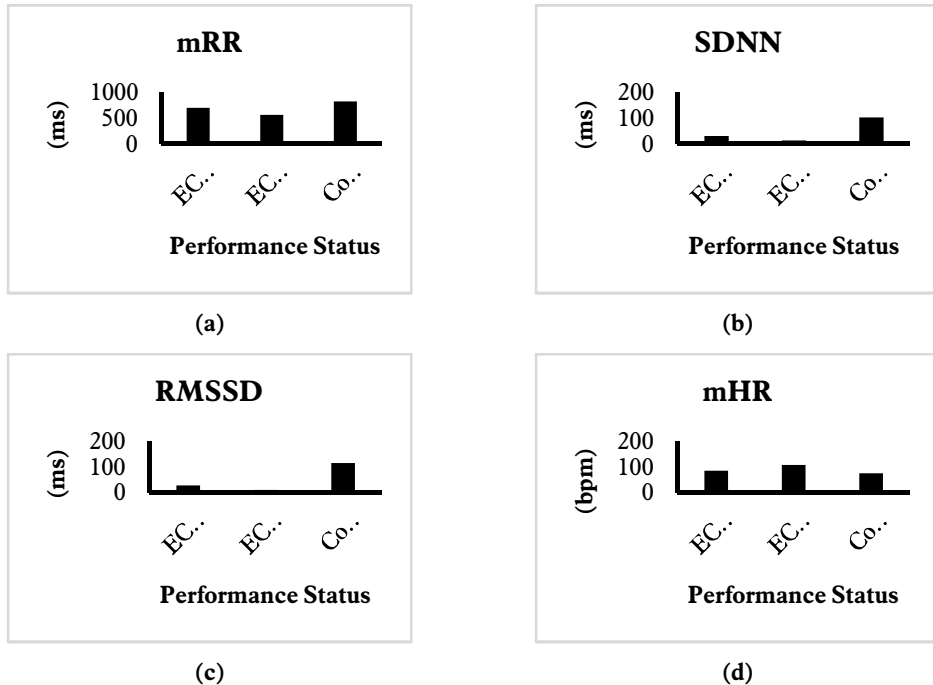
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Fig. 1: Time Domain Measures of Heart Rate Variability
(a) mRR (b) SDNN (c) RMSSD (d) mHR

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Classification Model for Predicting Disease Stage: ADHD Case Study

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ABSTRACT—*Attention deficit hyperactivity disorder (ADHD) is a neuropsychological disorder, which occurs in children at an early stage. This disease state is characterized by three main problems such as inattentiveness, hyperactivity and impulsiveness. Recent developments in machine learning have made significant impacts in the detection and diagnosis of various diseases. In this study, machine learning approach is used for improved diagnosis of the disease state among the above mentioned three symptoms. A set of 50 ADHD patient data was tested and trained by three of the machine learning models: Decision Tree, Random Forest and Neural Networks. A better prediction of the diseased state is obtained by the artificial neural networks with high accuracy. Further studies are in progress towards the optimization of parameters to increase the specificity and sensitivity of data using the ANN algorithm.*

Low Cost Foot Plantar Pressure Measurement System

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ABSTRACT—*One of the major complications caused due to poorly controlled diabetes is "Foot Ulcer". Infected diabetic foot ulcers account for approximately 25 % of hospital stays for patients with diabetes. A low cost device was aimed to identify patients who are likely to develop diabetic foot ulcer before its fruition. The device was designed to enable physicians to detect prospective areas of the foot for ulceration or its aggravation. Pressure exerted by the foot on the supporting surface during every day locomotive activities is known as "Foot Plantar Pressure". Real time monitoring of plantar pressure provides information that not only assists in detailing gait mechanics but also provides valuable insights to a variety of other disorders that support in detecting ulceration at its initial stages, which further aids in treatment and prevention of diabetic wounds. The literature review provided insights to the relation between foot pressure and its prospective risk of ulceration. The device was conceptualized to detect pressure areas of the foot when planted on a supporting surface. In the design and prototype development stage the device was fabricated with an appropriate sensor system integrated with a microcontroller. The data acquired from the system was further processed to compute a high resolution graphical image and a display in real time that provided an enhanced visual map of the areas prone to diabetic ulceration in the foot. The principle concept of foot plantar pressure monitoring provided effective and efficient information to detect foot ulcers or it aggravation before fruition. The low cost fabricated sensor system proved to be a design of precision having a sensitivity of a higher order and the visual map was found to be more accurate in comparison to the prevailing systems.*

KEYWORDS: *Foot Pressure, Plantar Pressure, Diabetic Foot, Foot Ulcer Detection, Diabetic Wounds Detection System*

BT

BIOTECHNOLOGY (ORAL PRESENTATION)

Identification of Microbial Lipase and Optimization of its Biocatalytic Activity

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ABSTRACT—*Lipases are glycerol ester belongs to the class of enzyme hydrolases that catalyze the hydrolysis of triglycerides to free fatty acids and glycerol over an oil–water interface. Microbial lipases have high industrial attention due to their stability, selectivity, and broad substrate specificity. In view of the increasing understanding of lipases and their many applications in high-value syntheses and as bulk enzymes, these enzymes are having an increasing impact. They are currently given much attention with the rapid development of industrial applications. Mangroves are the wetland forest highly influenced by the tides from the sea. They are inter-tidal regions between the land and sea typically found in tropical and subtropical regions. They are the rich source in microbial diversity including lipase producing bacteria. Soil sediment from mangrove site was collected in a sterile container. These samples were screened for a novel lipase producing bacteria by screening in Tributyrin agar plate, Rich medium and Rhodamine olive oil agar and liquid medium. Among the 68 isolates 25 isolates exhibited high lipolytic activity on the lipase screening liquid medium using olive oil as substrate. Lipase activity was further quantified at 410 nm spectrophotometrically. Molecular Identification of the bacteria will be Inferred by 16s RNA sequencing using 27F and 1492R/1390R/907R primer sets and analysed by BLAST. Characterization of various physicochemical, bio-chemical and micro-biological parameters are carried out and specific lipase activity will be assayed. The biocatalytic activity of lipase enzyme is checked by using various types of oil substrates. Extraction and purification of the lipase enzyme will be done by using advanced purification techniques. Further based on the result this lipase enzyme showing highest biocatalytic activity will be used as a catalyst for industrial applications.*

KEYWORDS: *Microbioal Lipase, Mangrove, 16sRNA, Biocatalytic Activity*

Self-Assembled Genistein Loaded Pullulan Nanoparticles: A Potential Carrier for pH Dependent Sustained Drug Delivery

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ABSTRACT—*The nanotechnology research with its never ending applications in a variety of scientific fields is ever expanding. The unique properties of nanoparticles make them ideal for drug delivery and drug targeting applications. For drug applications, nanoparticles offer the advantage of controlled release, therapeutic impact and targeted delivery. Pullulan, a linear homopolysaccharide of glucose, often described as α (1- 6) linked maltotriose has been used in this study as a drug carrier in nano fabricated form. The advantage of exploiting nano materials such as pullulan is its flexibility in preparation and engineering where it can be customized according to requirement, its specificity for target delivery, and its drug loading capacity. In this unique approach pullulan has been modified (Acetylation of pullulan is carried out using Motozato's method) to alter its hydrophilic character to form hydrophobic nano-molecules and has been characterized for evaluation of its various properties like Contact angle analysis, Particle size distribution, Scanning electron microscopic analysis (SEM), AFM analysis, Drug encapsulation efficiency, Thermo gravimetric analysis, Fourier Transform Infra-Red Spectroscopy (FTIR), pH dependent Structural analysis and In vitro Drug release profile etc. Genistein, a low cost, safe and biocompatible natural compound has been used here as model drug loaded into the so formed nanoparticles. The results showed promising results of the formed nano molecules with formation of stable molecules with a size of 39 nm range without the drug having hydrophobic properties for specific applications. Keeping in mind about the fact of reported properties of pullulan for its affinity for organ specific accumulation (liver) and the potential of Genistein for targeting malarial parasite, the so formed drug loaded Pullulan nanoparticles in this study have tremendous potential application as controlled drug delivery system with high loading efficiency and controlled release profile.*

INTRODUCTION

The nanotechnology research with its never ending applications in a variety of scientific fields is ever expanding. Medical applications of nanotechnology in specific have the potential to revolutionize both the diagnosis and treatment of diseases. Nanomedical approaches to drug delivery focuses on developing nano scale particles or molecules to improve drug bioavailability. Drug delivery focuses on maximizing bioavailability both at specific places in the body and over a period of time [1–4]. The unique properties of nanoparticles make them ideal for drug delivery and drug targeting applications. For drug applications, nanoparticles offer the advantage of controlled release, therapeutic impact and targeted delivery. The targeting capabilities of nanoparticles depend on the particle size, surface charge, surface modification and hydrophobicity. Also, the active ingredients included in many formulations are usually hydrophobic and therefore, display poor water solubility, which prevents contact with cells and tissues. Pullulan is a linear

homopolysaccharide of glucose that is often described as □ (1- 6) linked maltotriose, secreted primarily by strains of the fungus *Aureobasidium pullulans* which has been used as a drug carrier in nano fabricated form. Due to their stability and ease of surface modification polymeric nanoparticles like pullulan made from natural and synthetic polymers have received the majority of attention. Biodegradable polymers have been studied extensively over the past few decades for the fabrication of drug delivery systems. Considerable research is being directed towards developing biodegradable polymeric nanoparticles for drug delivery and tissue engineering. The advantage of exploiting nano materials such as pullulan is its flexibility in preparation and engineering where it can be customized according to requirement, its specificity for target delivery, and its drug loading capacity. However, despite the fact that pullulan has been in commercial production for more than 25 years; few of these potential uses have been widely adopted. The unique linkage pattern of pullulan contributes to provide the polymer with distinctive physical traits, including adhesive properties and the capacity to form fibres, compression mouldings, and strong, oxygen-impermeable films. Due to its non-toxic, non-immunogenic, non-mutagenic and non-carcinogenic nature recently there is an attempt to explore this polysaccharide for various biomedical applications including targeted drug and gene delivery and surface modification [5–12]. The unique properties of this polysaccharide are due to its characteristic glycosidic linking [13–15]. Pullulan is easily chemically modified to reduce the water solubility or to develop pH sensitivity, introduce functional reactive groups etc. so that it can be utilized for a chosen application. The other advantages of pullulan are that it is a non-ionic polysaccharide and is blood compatible, biodegradable non-toxic, non-immunogenic, non-mutagenic and non-carcinogenic. Pullulan is highly water soluble; hence for drug delivery applications, mostly hydrophobized pullulan is used as drug delivery carriers. These hydrophobized pullulan molecules can form colloidal stable nanoparticles upon self-aggregation in water with monodispersity [16,17]. Pullulan due to its film forming properties can entrap biological molecules and due to its excellent oxygen barrier properties these molecules remain stable with enhanced shelf-life. Pullulan has been exploited for its affinity towards specific organs like liver. Pullulan accumulates in the liver in significantly higher amounts than other water soluble polymers. This property of pullulan can be widely exploited for targeted drug/ gene delivery to liver. Genistein (GEN) is a major isoflavone in soy and red clove having potential beneficial effects and numerous biological actions, which have become a focus for research in a substantial way. An increasing number of studies have revealed that dietary isoflavones, including GEN, may act against large spectrum of life threatening diseases like cancer[18–22], cardiovascular complications [23–25], malaria infection and parasite cell cycle in liver [26,27], Alzheimer's

disease (AD) [28,29] etc., without exerting toxic effects. In this study pullulan has been modified through acetylation process to bring out its hydrophobicity characteristics and then subsequently formed nanoparticles have been loaded with drug Genistein and thoroughly characterized through contact angle analysis, Particle size distribution, Scanning electron microscopic analysis (SEM), AFM analysis, Drug encapsulation efficiency, Thermogravimetric analysis (TGA), Fourier Transform Infra-Red Spectroscopy (FTIR), pH dependent Structural analysis and In vitro Drug release profile.

MATERIALS AND METHODS

MATERIALS

Chemicals were analytical grade and used as received with- out further purification. Pullulan (MW 200,000, PDI 1.53), Genistein was purchased from Sigma Aldrich. All glassware used in the laboratory experiments was cleaned with a fresh solution of HNO_3/ HCl (3:1, v/v), washed thoroughly with doubly distilled water, and dried before use.

METHODS

Synthesis of Nanoparticles

Acetylation of Pullulan: Pullulan is a hydrophilic polysaccharide by nature, thus it is very essential to change the hydrophilic nature into a hydrophobic for its applications in the controlled drug delivery system. To achieve this a proton ion of pullulan at glucose was substituted into acetyl group to prepare hydrophobic pullulan acetate. For the synthesis of Pullulan acetates Motozato method was taken into consideration [30], where optimization was done according to requirement. Pullulan was suspended in formamide solution and dissolved by vigorous stirring. After that pyridine with acetic anhydride were added to this suspension which was stirred for 48hrs with heating. Finally a dark brown precipitate was formed. The synthesized precipitate PA (Pullulan acetate) was extracted after reprecipitation. The solid material was vacuum dried to obtain powder form. The pullulan acetate achieved by this process was confirmed further by the use of FT-IR spectroscopic analysis.

Preparation of PA Nanoparticles: Pullulan acetate nanoparticle was prepared using the solvent evaporation method. Pullulan acetate was weighed accurately and dissolved in acetone and kept in beaker with continuous mild stirring. This was added to distilled water drop by drop by means of a syringe positioned with the needle directly under moderate magnetic stirring. After adding the solution in beaker was continuously stirred until the solvent get evaporated. This process helped in formation of nanoparticles.

Preparation of Genistein-PANP'S: Genistein and various amounts of PA were dissolved separately in of acetone, the solutions were stirred using magnetic stirrer needle directly in the medium under moderate 50 rpm. This was added to 20 ml of distilled water drop by drop by means of a syringe positioned with the needle directly under moderate magnetic stirring. After adding the solution in beaker was continuously stirred until the solvent gets evaporated which leads to the formation of nanoparticles. Nanoparticles were prepared in the drug: polymer ratio 1:2, 1:3, and 1:4 respectively to compare the drug loading efficiency.
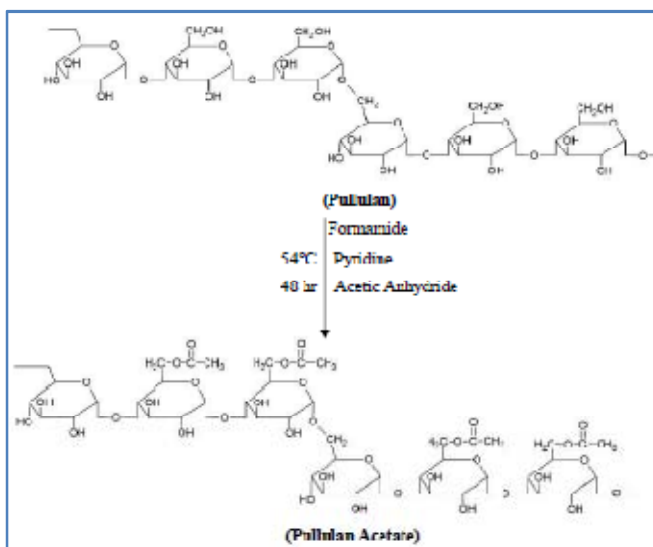
Fig. 1: Synthesis of Pullulan Acetate from Pullulan by Acetylation

Characterization of Nanoparticles

Contact Angle Analysis: Contact angle measurements were performed with the help of Holmarc Contact angle meter, India to investigate the hydrophilic character of the functionalized nanoparticle surfaces. The pictures obtained with the CCD camera and the corresponding angles are shown in Fig. 2.

Particle Size Analysis: The mean particle size and size distribution of each batch of nanoparticle suspension and drug loaded nanoparticle was analyzed using the Zetasizer analysis (Brookhaven instruments, USA).

Morphological Study of Nanoparticles

Scanning Electron Microscope: The morphology of the nanoparticles was observed using an SEM [Zeiss gemini, Germany]. A drop of the nanoparticle suspension

(5–10 μL) was placed on a silicon wafer surface and air dried at room temperature. The sample was then coated with gold/palladium by Ion Sputter. Coating was performed at 20mA for 4 min, and observation was made at 25kV.

AFM Measurements: Morphological evaluations of Pullulan nanoparticles were realized with an Atomic Force Microscop.

Encapsulation Efficiency: An accurately weighed quantity of the primary nanoparticle solution was centrifuged at 6000 rpm for 30 min. The dispersion was filtered and the absorbance of the filtrate was measured at 421 nm after appropriate dilution in a UV-visible spectrophotometer (Eppendorf Biospectrometer). The drug content was estimated in triplicate using a calibration curve constructed in the same solvent. Polymers did not interfere with the assay at this wavelength. The amount of drug encapsulated in to the microspheres is determined by using the following formula.

The drug encapsulation efficiency (EE) of the microspheres was calculated as follows

Total amount of drug–Free drug

Encapsulation efficiency = $\times$ 100

Total amount of drug

Thermogravimetric Analysis: Thermogravimetric analysis (TGA) was done using thermogravimetric analyzer (Perkin Elmer STA6000). Samples were heated from 0 to 800 °C in a platinum pan with a heating rate 20 °C/min, in N2 atmosphere.

Fourier Transform Infra-Red Spectroscopy (FTIR): Drug polymer interactions were studied by FTIR spectroscopy. The FTIR spectra of drug, pullulan and drug loaded pullulan nanospheres were determined by using Perkin-Elmer FT-IR model. The pellets were prepared by gently mixing of 2mg sample with 200mg potassium bromide. The scanning range was 500 to 4000 cm^{-1} and the resolution was 4 cm^{-1}.

pH Dependent Structural Analysis: To understand the effect of pH on the so formed nanoparticles, samples were kept in different pH solutions for 24 hrs. in a Nitrocellulose membrane. Then the samples were collected from the membrane and taken for SEM analysis for determining the effect of pH on the structure of so formed nanoparticles.

In vitro Release Studies: The release study of the drug loaded microspheres was carried out in the following manner; 25 mg of Genistein-loaded microspheres in 1 ml of 0.15M phosphate buffered saline (PBS; pH 7.2 and 4.0) were put into two different dialysis tube which was introduced into 100 ml of respective buffer and was

kept in a stirrer at gentle rpm at room temperature. At specific time intervals, the released sample was taken out to find the concentration of the released drug and replaced respectively with freshly prepared buffer. The concentration of the released drug was estimated by using UV spectrophotometer at 421nm.

RESULTS AND DISCUSSION

Contact Angle Analysis

The initial clean surface indicates the highly hydrophilic nature of Pullulan powder a. Upon acetylation of pullulan the hydrophilic property of formed Pullulan acetate decreases significantly and was measured to be at 60°. The drug loaded pullulan acetate nanoparticles showed similar lesser hydrophilic characteristics at angle of 54°. The acetylation of pullulan resulted in this reduction in hydrophilic property giving pullulan acetate a hydrophobic tendency which retains even after successful drug loading.
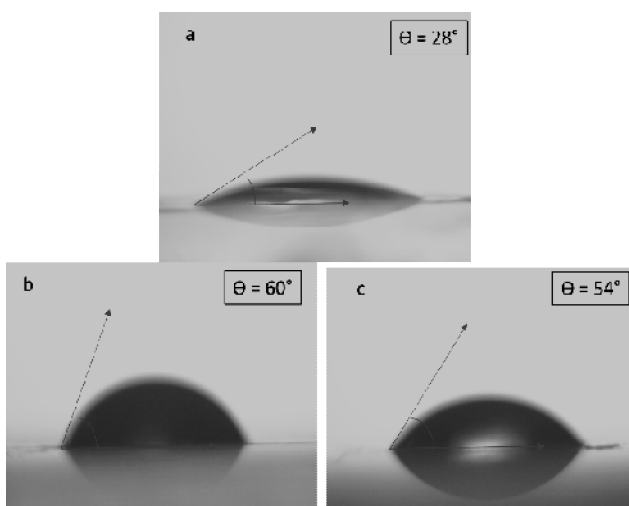
**Fig. 2: Static Water Contact Angles Pictures, and Corresponding Angles;
a. Pullulan Powder, b. Pullulan Acetate Nano-Particle,
c. Drug Loaded Pullulan Acetate Nanoparticle**

Particle Size Analysis

The size distribution profile, as shown in Fig 3 , represents a typical batch of bare pullulan acetate nanoparticles with a mean diameter of = 38.45 nm whereas the mean diameter of Genistein-loaded nanoparticles was about 105.02 nm as shown in Fig. 3 Drug loaded nanoparticles were stable under the autoclaving conditions.
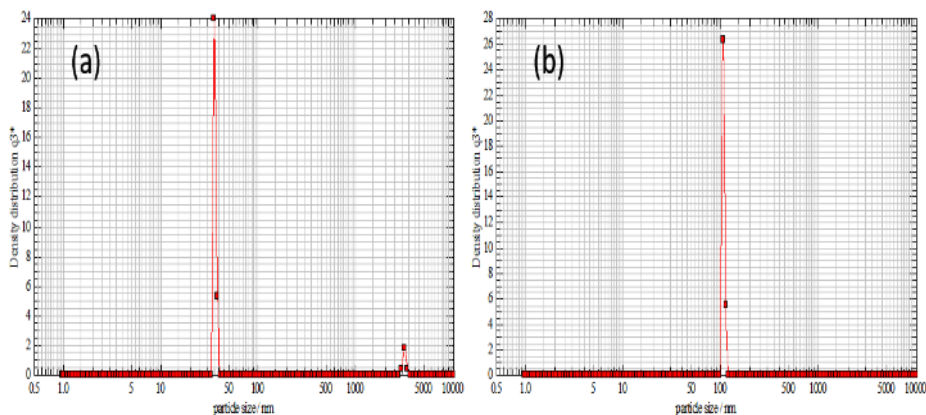
**Fig. 3: Particle size Distributions of Pullulan Acetate Particles
(a) and Genistein Loaded Pullulan Acetate Nanoparticles (b)**

Morphological Study of Nanoparticles
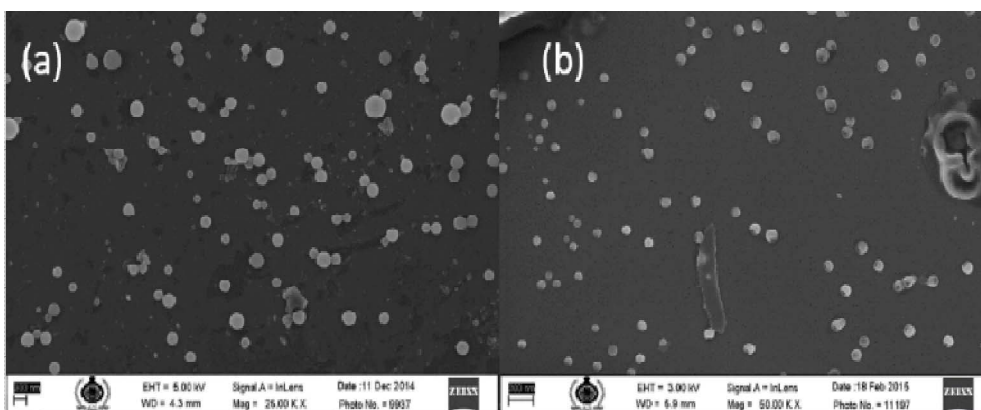
Scanning Electron Microscope

**Fig. 4: Scanning Electron Microscopy (SEM)
Photographs of Pullulan NPs (a), Drug Loaded Pullulan NPs (b)**

SEM micrographs (Fig. 4) show that most of the particles are spherical in shape. Considering that Pullulan NPs has been synthesized by wet soft chemistry, the uniform size and shape are achieved. The SEM of the drug loaded chitosan microspheres showed that the nanospheres have a solid dense structure with smooth spherical shape. Also the SEM analysis corroborate with that of the finding of size analysis done by particle size analyzer.
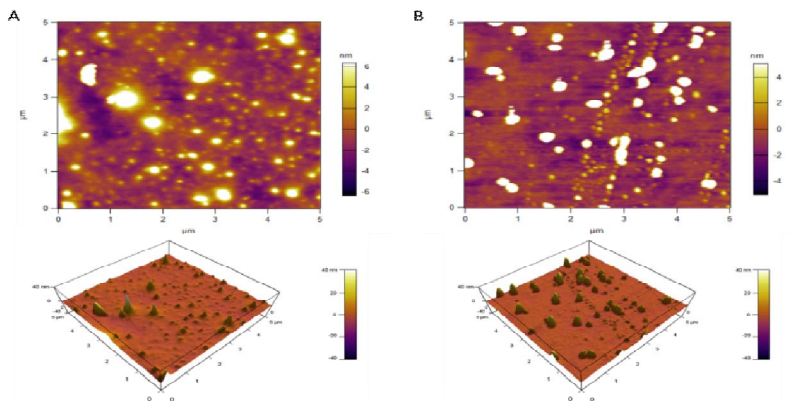
AFM Measurements

**Fig. 5: Atomic Force Microscopy Images of Pullulan
(A) and Genistein Loaded Pullulan (B)**

The AFM results display the surface morphology of the monodispersed Drug loaded Pullulan NPs formed. As observed in Fig. 5, the value determined by the AFM was close to that determined by the SEM, and the particles of pullulan NPs and drug loaded Pul NPs showed a dense and uniform packed structure.
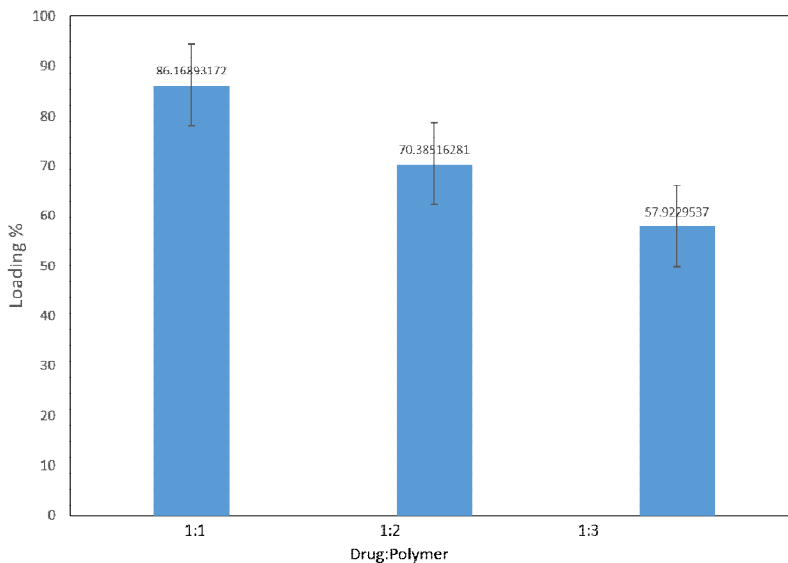
Encapsulation Efficiency

Fig. 6: Encapsulation Efficiency of Various Drug: Polymer

The results indicate that the encapsulation efficiency is a drug to polymer ratio dependent process. The ratio 1:1 seemed to be the best option (86.16%) for highest loading efficiency compared to other ratios tested (Fig. 6).

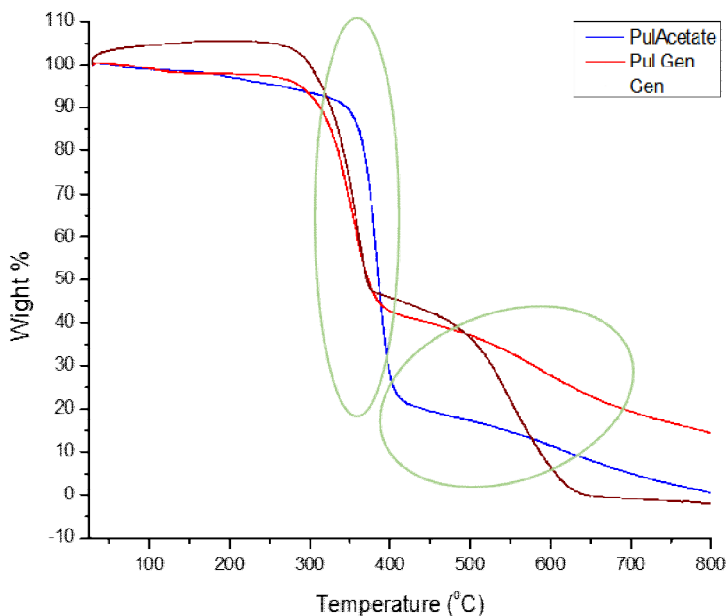**Thermogravimetric Analysis**

Fig. 7: Thermogravimetric analysis (TGA) of Naked Pullulan Acetate Particles, Genistein and Genistein Loaded Pullulan Acetate Nanoparticles

TGA was performed to confirm the coating formation and to estimate the content of drug in the Pullulan Acetate Nanoparticles. The DSC thermogram patterns of Genistein, Pullulan and Genistein loaded Pullulan particles are shown in (Fig. 7). The weight loss pattern was similar for all samples. The first loss of 80% for Pullulan acetate, 48% for Genistein and 42% for Pullulan Genistein happened from room temperature to 400°C. A second loss of weight was observed at around 600°C. When we compared TGA in samples, the main difference is that pullulan references initially loss more weight than those nanomaterials with drug; this can be explained due to the time of aging in both samples, since drug-loaded pullulan required higher time than pullulan alone.

FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FTIR)
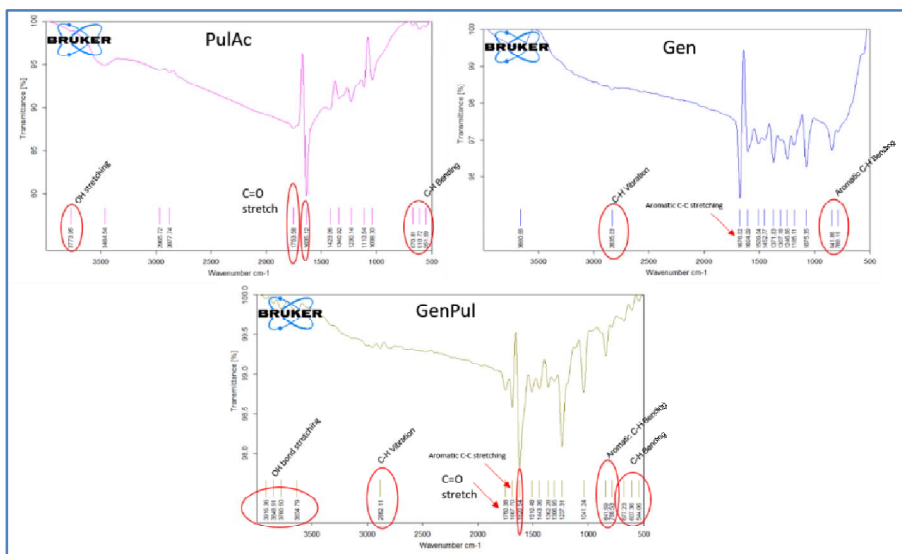

**Fig. 8: FTIR analysis of Naked Pullulan Acetate particles,
Genistein and Genistein loaded Pullulan Acetate Nanoparticles**

The FTIR spectra of Pullulan acetate clearly projects the signature addition of acetate group with introduction of C=O stretch at 1750 cm^{-1}, CH$_3$ deformation at 1370cm^{-1}, O–C=O bend at 602 cm^{-1} and CH bend at 610cm^{-1}.Genistein shows its signature patterns with aromatic CC stretching at 1676 cm^{-1} and aromatic CH bending at 841 cm^{-1}. The conjugated compound shows no changes in peak signatures indicating no major interaction between drug and polymer. It is thus suggested that the interaction is mere encapsulation of drug inside the so formed particles with mere light hydrogen bonding or Van-dar wals force.

pH DEPENDENT STRUCTURAL ANALYSIS

The SEM images (Fig. 9) provided information regarding the overall particle structure which remained spherical but with distinct swelling of nanoparticles compared to initial state upon incubation in different pH solutions. The result clearly indicated that with increasing pH the swelling of formed nanoparticles was increased. It was observed that at pH 7.2 the swelling was moderately optimum compared to lower pH values tested and the structure retains comparatively better than both lower and higher range.
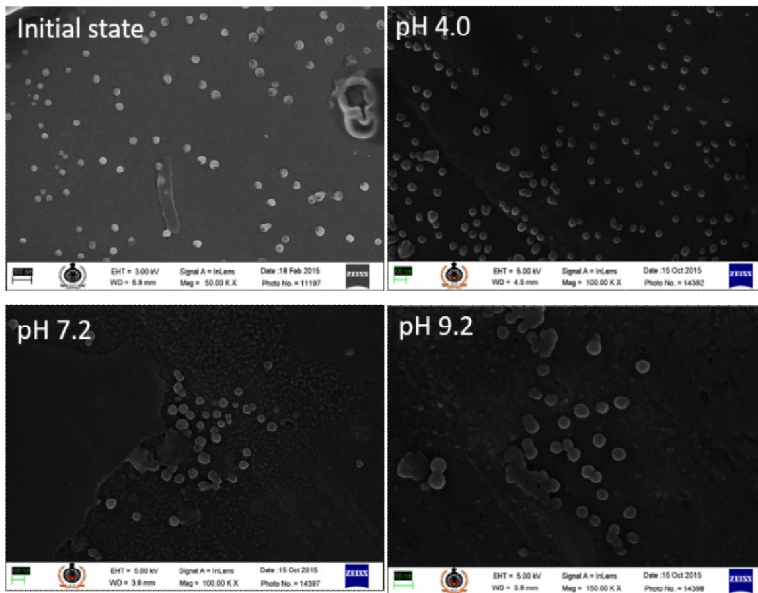
Fig. 9: SEM Images of Pullulan Acetate Nanoparticle in Different pH solutions
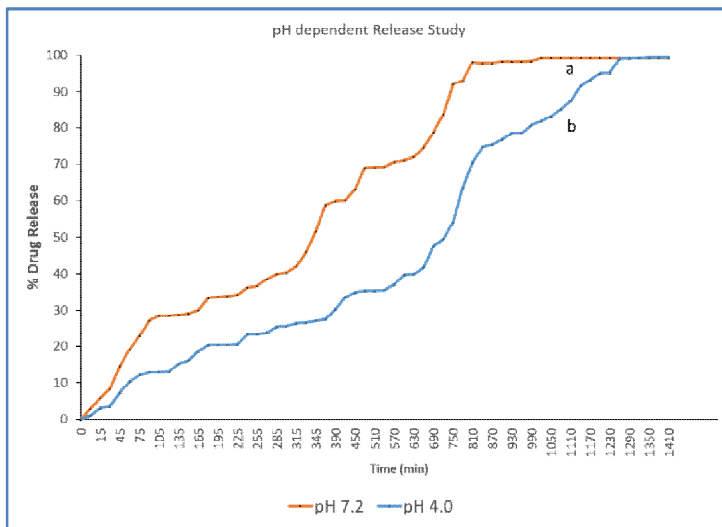
IN VITRO RELEASE STUDIES

Fig. 10: In-vitro Release study of Genistein-Loaded Pullulan Acetate Nanoparticles at pH 7.2 (a) and pH 4.0 (b)

The release pattern for Genistein-loaded nanoparticles at different pH solutions are shown in Fig. 10. Genistein released from the nanospheres was much faster at pH 7.2 than that at pH 4.0.The possibility is that the solubility of drug is dependent upon the pH of the PBS buffer. There also may be other possibility that the swelling of pullulan acetate depends upon the pH-sensitive factor. At pH 4.0, the carboxylic groups of pullulan acetate were protonated due to which the pullulan acetate microspheres did not swell enough. Thus Genistein was not released from the pullulan acetate microspheres. In contrast, at pH 7.2 the amount of Genistein released increased generally because the microsphere swelling enhanced effectively due to the ionization of carboxylic groups at a neutral pH. Therefore, the initial release of Genistein was started by the pH-sensitive diffusion of drug contained at the microsphere surface, then this release phase was followed by the pH-sensitive swelling behaviour of microspheres.

CONCLUSION

This work demonstrates the potential of drug conjugated Pullulan nanoparticles in the preparation and drug loading efficiency which can be tuned as per requirement. The self-assembled hydrophobic nanoparticles were prepared in the size range of 38.45 nm. The formed particles have been tested to be stable and the acetylation of pullulan has not altered its property to form hydrophobic nanoconjugate ready for drug loading. The pH dependent controlled drug delivery proficiency of pullulan nanoparticles showed highly positive results indicating a potential drug carrier system with controlled release mechanism for longer/slower effect on target site. Also the capability of such formed nanoparticles to deliver drug at desired location too is a high possibility which further need to be tested. Overall this study clearly showed possibilities of exploiting the potential of pullulan mediated pH dependent controlled drug delivery in a time dependent fashion and seek for further studies to be made to explore more in this promising area.

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Comparative Studies on Production of Statins using Three Different Microbial Strains in SSF Statins Production by High Yielding Strains

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ABSTRACT—*The present study aimed to increase the yield of mevastatin and lovastatin in solid state fermentation (SSF) using three different fungi, Aspergillus terreus MTCC 279, Penicilliumcitrinum MTCC 1751 and Penicilliumbrevicompactum MTCC 549. Initially, various substrates were screened for maximizing mevastatin and lovastatin production. Barley powderwas found to be a most suitable substrate for A. terreus, as it gave the maximum mevastatin of 272.16 mg/gram dry substrate (gds) and lovastatin of 206.8 mg/gds. The high yielding strain of A. terreus was used for further studies with barley as substrate. Response surface methodology (RSM) was applied to determine the optimal parameters for initial moisture content, temperature and inoculum size. In validation experimentation, the maximum amount of mevastatin (297.98 mg/gds) and lovastatin (340.71 mg/gds) was obtained using A. terreus with barley as substrate. This is the first report on the simultaneous production of drugs mevastatin and lovastatin using high yielding strain in SSF.*

KEYWORDS: *Solid State Fermentation, Mevastatin, Aspergillus Terreus, Penicilliumcitrinum, Penicilliumbrevicompactum, Response Surface Methodology*

Microbial Keratinase and its Multi-Functional Biocatalytic Applications

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ABSTRACT—*The ability of Bacillus licheniformis (MCC2830) to utilize different keratin substrates (soluble keratin, hair, feather, nail and scales) was tested along with dehairing capacity. The keratinases showed high substrate specificity for soluble keratin, fish scales and chicken feathers and nails, but low specificity for human hair. The enzyme is heat stable (37-45°C) and active around wide pH range (2–8). The bacteria completely degraded whole chicken feathers after 5 days at 37 ± 2 °C, and also completely dehaired goat skin within 9 hrs, without damage to the skin. The crude extracellular keratinase obtained from a keratin-degrading bacterial strain, showed a remarkable ability to synthesis silver nanoparticle (AgNPs) within 2 hr. Therefore, the keratinase of Bacillus licheniformis strain could be used to develop an environmental friendly method for bioconversion of waste feather into feather feed, for dehairing process in leather industry & rapid biosynthesis of silver nanoparticles.*

KEYWORDS: *Microbial Keratinase, Bioconversion, Feather Feed, Dehairing, Silver Nanoparticles, Green Synthesis*

Development of Chitosan based Films from Shrimp Waste

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Objective: *Development of chitosan based films from shrimp waste*

Methodology: *Extraction of chitosan from shrimp waste was carried out using NaOH and Solubility of extracted chitosan was analyzed at 0.5 & 1% concentration of different solvents like acetic acid, formic acid, lactic acid and propionic acid. Different properties of developed film such as mechanical and antimicrobial were analyzed for various concentration of chitosan from 0.5 to 2%. To check the potential of developed film further it was studied for preservation of fruits and vegetable.*

Results and Conclusions: *To achieve the maximum Degree of De-acetylation i.e. 90%, different concentration of NaOH from 40 to 70% was used and 70 % NaOH was optimized. Among all solvents 1% concentration of acetic acid was found to be the best for solubility. Best results with respect to its properties like tensile strength, thickness and % elongation was obtained with addition of 2% chitosan. Resulted film showed maximum resistance to water vapour permeability and microbial activity against foodborne pathogens i.e. Staphylococcus aureus and Pseudomonas aeruginosa. Further to check its potential tomatoes and apples were packed in chitosan based film during storage. With compared to control sample, shelf life of tomato and apple has extended upto 8 days at $30\pm2\ ^oC$.*

Intranasal Mice Model to Study the Role of *Bordetella Pertussis* Antigens in Immunity

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Yojana Shinde[4], M. Anand Kumar[5] and Deepti D. Deobagkar[6]**

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ABSTRACT—*Pertussis known as whooping cough is a highly contagious disease. Whole cell pertussis vaccine is the most economical and effective strategy for preventing and controlling pertussis. The efficacy of whole cell vaccine is ascertained most commonly by intracerebral challenge assay, but it do not reflect the true efficacy of vaccine as Pertussis essentially is a respiratory disease. Therefore to mimic the natural infection intranasal challenge model in mice is developed. In intranasal challenge assay mice are immunized with vaccine and challenged through intranasal route using B. pertussis 18323 strain. Mice lungs are dissected and examined for B. pertussis count and the degree of count is related efficacy of vaccine. Higher count indicates low efficacy and low count points to better efficacy.*

Whole cell pertussis vaccine should contain agglutinogen 1, 2 and 3 to produce vaccines with very high efficacy. In the present study the role of Bordetella pertussis agglutinogen 1 in protection against infection is investigated in intranasal challenge assay, where a whole cell vaccine formulation using B. pertussis strain 353, which contain only agglutinogen 1 is prepared. Mice are protected against infection as indicated by lung clearance. But when the protection level is compared to other B. pertussis vaccine strains which contain agglutinogen 2 and/or 3 in combination with agglutinogen 1, an inferior protection is observed. This clearly indicates that the presence of agglutinogen 2 and/or 3 in combination with agglutinogen 1 results in better protection. Moreover the lung clearance observed with agglutinogen 1 may be attributed to the presence of other antigens as whole cell is used for preparing the formulation.

Discovering New Microbial Metabolites using Novel Methodologies: A Special Instance of Antitumor Antibiotic

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ABSTRACT—*Microbial metabolites have long history as lead compounds for discovery and development of new drugs in different diseases. Environmental microbes, particularly actinomycetes have an exceptional ability to synthesize metabolites with tremendous pharmacological relevance.*

*Undiscovered species inhabiting unique environments are thought to be sources of novel chemical scaffolds. Phylum Actinobacteria has a long history in the treatment of TB. For increasing discovery rates of new structures, we intensified our searches towards some unexplored habitats for isolation and evaluation of potential antagonistic actinobacteria. In this course, a total of 125 actinobacteria were isolated from various soil samples from untapped areas in Northwestern Himalayas, India. The antibacterial screening showed that 26 isolates inhibited the growth of at least one of the tested bacterial. The screening of fermentation products from the selected 26 bioactive isolates revealed that 10 strains have metabolites antagonistic against the standard H37Rv strain of Mycobacterium tuberculosis with their MIC values ranging between · 1.95 to 250 μg ml^{-1}.The biosynthetic metabolites from isolates viz AS008 and AS013 exhibited promising anti-tubercular activity with least MIC's of 3.9 and <1.95 μg ml^{-1}. Higher bioactivity at the preliminary screening and lack of any report regarding the chemoprofile of Lentzea violacea strain AS08, the strain was selected for further investigation and its culture optimization was carried by developing novel method for dereplication. A new eudesmane sesquiterpenoid (**1**), and a new homologue of virginiae butanolide E (**2**) along with butyl isobutyl phthalate (**3**) were isolated from, actinomycete- Lentzea violacea AS08 by stressing on modified one strain-many compounds (OSMAC) method. The compounds 1 and 2 showed moderate activity against gram negative (MIC~32-64 μg ml^{-1}) in comparison to gram positive bacterial pathogens. Compound 1 exhibited significant activity in human cancerous cell lines (IC$_{50}$ 19.2μM).*

*Streptomyces puniceus, was next searched for production of bioactive metabolites and bioassay-guided fractionation of microbial cultured of its ethyl acetate extract led to isolation of macrotetrolide compound **1'** (Dinactin) and compound **2'** (1-(2,4-dihydroxy-6-methylphenyl)-ethanone). Compound 1' exhibited strong anti-microbial activity against all tested bacterial pathogens including Mycobacterium tuberculosis. The MIC values of compound 1' against Gram negative and Gram positive bacterial pathogens ranged between 0.019 - 0.156 μg ml^{-1} and 1 μg ml^{-1} against Mycobacterium tuberculosis H37Rv. Dinactin exhibited marked anti-tumor potential with IC$_{50}$ of 1.1- 9.7 μM in vaous human cancerous cell lines and showed least cytotoxicity (IC$_{50}$ ~ 80μM) in normal cells (HEK-293). Dinactin inhabited cellular proliferation in cancer cells, reduced their clonogenic survival as validated by clonogenic assay, also inhabited cell migration and invasion characteristics in colon cancer (HCT - 116) cells. Our results expressed the antimicrobial potential of dinactin and also spotted its prospective as an antitumor antibiotic. Further these studies supported that isolation of new molecules by the exploration of new strains and intensifying methodologies are promising approaches towards discovery of novel lead molecules*

KEYWORDS: *Microbial Metabolites, Lentzea Violacea, Streptomyces Puniceus and Novel Molecules, Antitumor Antibiotic*

Transcriptomics Analysis of *Bacillus Cereus* during Biosynthesis of Selenium Nanoparticles

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ABSTRACT—*In the present study selenium nanoparticles synthesized by using Bacillus cereus strain isolated from western ghat of Maharashtra with high level of tolerance to selenite (up to 50mM). Isolated Bacillus cereus when grown in the presence of selenite accumulates intracellular deposits of red selenium nanoparticles giving culture media an orange to dark-red colour. The as-prepared selenium nanoparticles have been characterized by XRD, UV-Vis absorption spectroscopy, FT-IR, SEM and TEM. Here, we highlight possible selenium biomineralization mechanism through analysis of the global transcription changes by Illumina next generation sequencing. Transcriptomics analysis indicated that 42 genes were upregulated and 54 were downregulated in the cells. The majority of genes related to transcription, translation, oxidative stress, cell adhesion, cell wall biosynthesis were upregulated, while genes belonging to membrane transporter activity, amino acid metabolism, cell wall biosynthesis were downregulated. Overall, this study presents a useful picture of identification of candidate genes involved in selenium nanoparticles biosynthesis.*

Arachidonic Acid and Docosahexanoic Acid Stimulated Recovery of Haematopoiesis in Hematopoietically Compromised Mice

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Stem cell therapy has great potential to treat of numerous degenerative diseases and holds promise for regenerative medicine. To control stem cell fate towards proliferation or differentiation is a key factor in the therapy. So far, the safe effective and practical means to control stem cell fate were not successfully fond. Arachidonic acid (AA) and Docosahexanoic acid (DHA) are essential polyunsaturated fatty acids (PUFAs) from omega 6 and omega 3 group respectively. They are critical components of cell structure and function and have shown to influence stem cell fate. Being natural compounds, they can be used as nutritive supplements in regenerative medicine. Very few reports talk about their effect on hematopoiesis. Earlier we have shown that that oral administration of PUFAs including AA and DHA enhance haematopoiesis in healthy mice. In this study, AA and DHA showed superior enhancement than their parent PUFAs. Next we wanted analyze whether AA and DHA would have similar effect in hematopoietically compromised condition. In this study we irradiated mice to cause their bone-marrow (BM) depletion. These mice were then fed with AA or DHA and later their BM cells and peripheral blood (PB) cells were subjected to assays for haematopoiesis. We observed that PUFAs significantly enhanced total nucleated cells (TNCs), lineage negative cells, leukocytes especially lymphocytes in mice. To further evaluate clinical relevance of our findings, we studied the effect of feeding with AA/DHA on stimulation of haematopoiesis in bone marrow transplanted (BMT) mice. The beneficial effect of AA/DHA was also observed in bone marrow-transplanted mice. Significant increase in the TNC count, number of RBCs, platelets and lymphocytes was observed in the BMT recipient mice fed with AA/DHA. These mice also showed significantly higher long-term engraftment in the bone marrow as well as peripheral blood cells of BMT recipient mice indicating that AA/DHA simulated homing of haematopoietic stem cells in recipient mice. Taken together, PUFAs enhanced haematopoiesis and thrombopoiesis in irradiated mice and help in recovery of BMT mice. Our novel findings will pave ways for incorporating AA/DHA as a dietary supplement in treatment of hematological malignancies, leukemia, chemo/radiotherapies and bone marrow transplantations.

Fate of Clinical Listeriosis in the Indian Subcontinent

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Listeria monocytogenes is a gram positive pathogenic bacterium that can cause listeriosis, A severe invasive infection in humans. They are the most important causes of death from food borne infections with the third highest mortality rate among food borne pathogens after Salmonella Campylobacterand Clostridium. It is a gram positive bacterium that belongs to the category of pathogens that escape from an internalization vacuole and proliferate in the cytosol of mammalian cells It thrives across a range of temperature, salinity and pH.It is ubiquitous in its distribution and have been isolated from sources such as soil, water, animal sources, raw milk, ready to eat deli meats, fresh plant and animal produce and refrigerated smoked food. There are 18 species and 13 serotypes of listeria that are reported till date and only serovar 4b/,1/2a and 1/b are known to cause over 98% of the human listeriosis. In most of the cases it's the consumption of contaminated food products is the main route of transmission, including the recent listeriosis outbreak in South Africa.

It generally affects pregnant women, those in extremes of age, i.e. new born and people above the age of 65 and also people with comorbid diseases such as diabetes mellitus, immunosuppression and HIV and liver disease. Although isolated since 1924 listeria and associated infections still remain a major challenge to the scientific community. It is ranked in the top 5 food borne pathogens among the list of 31 food borne pathogens listed by CDC, USA.

In India very few cases of clinical listeriosis have been reported. Among the reports that that exists studies are limited till isolation and characterization. These studies have been restricted to only a limited number of samples and geographical region hence the data is non conclusive.

According to the Indian listeria culture database, studies on Molecular epidemiology of listeria are lacking. We are underestimating the genetic variability of the strains isolated and also its epidemic potential. This work focuses on the state of listeriosis in the Indian subcontinent specially with reference of clinical cases of listeriosis in Humans. Isolation of listeria from CSF, Blood, amniotic fluid and placenta are considered to be of gold standard in the diagnosis of listeriosis. It also elucidates the lacunae in the study of listeria and the reasons for it being underdiagnosed and ignored in the Indian context in an attempt to draw the attention to a pathogen that quite arguably remains a great threat to the safety of our food supply.

BI
BIOINFORMATICS (POSTERS PRESENTATION)

In Silico Characterization of PBP2a Involved in Peptidoglycan Synthesis in Multi Drug-Resistant *Staphylococcus aureus*

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ABSTRACT—*Methicillin-resistant Staphylococcus aureus (MRSA) is a versatile pathogen capable of causing a wide variety of human diseases. The Meca gene is a gene found in bacterial cells and, that allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics. The Meca gene encodes of 668 amino acid residues which is a different form of low-affinity penicillin-binding protein (PBP2a), which β-lactam drugs cannot inactivate, thus allowing normal cell wall synthesis to occur even in the presence of these drugs and has been identified in various staphylococcal species, such as S. aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus, and Staphylococcus fleurettii. The Meca genes present in these species have >98% sequence identity with the mecA carried by the first fully sequenced prototype MRSA strain N315. In the present study Primary protein structure analysis of PBP2a by Prot Param revealed that theoretical pI value was 8.70 and molecular weight 76102.53. Aliphatic index was 82.43 and GRAVY -0.708. P fam domain analysis showed that PBP2a protein has MecA_N (NTF2-like N-terminal Transpeptidase domain)-25 to 140 aa, PBP_dimer (Penicillin-binding Protein dimerization domain)-146 to 313 aa. Transpeptidase (Penicillin-binding Protein Transpeptidase domain)-345 to 658 aa. Homology modeling of PBP2a was carried out using PDB ID: 1VQQ as a template and model quality 91.5% was observed. PBP2a considered as a potential drug target to design new inhibitor against multi drug-resistant Staphylococcus aureus.*

KEYWORDS: *In Silico, PBP2A, Meca, Peptidoglycan, Multi Drug–Resistant, Staphylococcus Aureus*

Identification of Inhibitor Against *H. Pylori* HTRA Protease using Structure-based Virtual Screening and Molecular Dynamics Simulations Approaches

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ABSTRACT—*Helicobacter pylori efficiently colonize at the gastric epithelial of the host cells and disrupt the mucosal integrity of E-cadherin by spreading several inflammatory diseases like chronic gastritis, gastric malignancies, gastric cancer, etc. The secretary HtrA protease recently reported as a novel therapeutic target for gastric cancer. The inhibition of HtrA-mediated cleavage of E-cadherin significantly reduces the migration of H. pylori infections. The limited knowledge about molecular diversity, structural and functional behavior of HpHtrA necessitated to explore the inhibition mechanism. Therefore, in this study, the similarity of HpHtrA protease with other gastrointestinal pathogens as well as a distant relationship with human HtrA homologs were studied by the phylogenetic analysis and hence was considered as a novel target for inhibitor designing. Due to the lack of crystal structure, the three-dimensional structure of HpHtrA was modeled and validated by SAVES, Verify 3D and ProSA server. The stable conformation of HpHtrA was identified through molecular dynamics simulation and was used for virtual screening to identify possible lead compound from the pool of ~1.3 million small molecule database. From virtual screening, top three potential lead compounds were further subjected to molecular dynamics simulation for 50 ns and binding free energy analysis. Among the top three compounds, the compound ID 300040 shows a better affinity towards HpHtrA. It is also important to mention that the ligand binding towards the catalytic site of HpHtrA is mainly facilitated by the L2 loop. The identified lead molecule having hydroxyl-piperidine with 4-aminopiperidine scaffold (which already known for its anti-cancerous properties) would help in the drug discovery process against H. pylori.*

KEYWORDS: *HPHTRA, Homology Modeling, Molecular Dynamics, Virtual Screening, Binding Energy, Hydroxyl-Piperidine, 4-Aminopiperidine Scaffold*

Molecular Dynamics and Lead Evaualtion of Mutated α–Synuclein Mis-Aggregation in Parkinson Disease

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ABSTRACT—*Parkinson's disease is the second most expanding neurodegenerative issue. Benchmark examine demonstrated that event of Lewy body development and its self-conglomeration is the charge of its harmful impact on dopaminergic neuronal cells. Also, the pH of the alpha-synuclein and the mutations, the gene responsible has an emendable effect on the aggregation and formation of fibril resulting in the following disease. Consequently the present review is aimed at deciding a medication competitor that can repress the self-total of Alpha-Synuclein in view of structure based medication outline. We tried to find out the aggregating pH previously in the research and currently on mutations. The three known mutations along with the new screened mutations prove to have more aggregating capacity. A53T, E46K, A91D, G41D, and A30P, identified from predict SNP as deleterious. The E46K mutation of SNCA changes the polarity of alpha synuclein and affects the occurrence of significant physico-chemical and molecular changes in this protein. It has also been suggested, that the E46K mutation may affect the release of neurotransmitters and lead to a more effective aggregation of alpha synuclein compared to the A53T and A30P mutations by altering the binding of alpha synuclein with the phospholipids of cell membranes. The currently found two mutations, i.e., G41D and A91D have proven to be responsible for the misfolding and can be a potential cause for the disease. Therefore our research aimed at studying the dynamics of the combinations of mutation which has resulted in a misfolded protein through Discovery Studio and Deshmond. The ability of the identified drug to bind to the active site of the protein was studied through docking. And the effectiveness of the drug to provide neuroprotection was obtained through molecular dynamics for 50s. It is been proved that the new mutation is immensely responsible for causing aggregation, and the identified drug prevents the misfolding.*

KEYWORDS: *SNCA, Alpha-Synuclein, Lewy Bodies, Mutation*

BM

BIOMEDICAL (POSTERS PRESENTATION)

Design of a Two-Way Adjustable Clubfoot Corrective Splint

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ABSTRACT—*Congenital talipes equinovarus, or clubfoot, is a common deformity characterized by inwards rotation of ankle in one or both feet. The prevalence rate of clubfoot in developed countries is about 1.2 per 1000 births whereas in developing countries like India, it is about 1 in 500 out of which 80% are left untreated due to unawareness. This deformity can be corrected by bracing and manipulation. Once diagnosed at birth, infants require immediate treatment to prevent lifelong disability. Ponseti method used widely consists of using six to eight different heavy casts each worn for about a week. A 3D scalable model of splint/brace was designed and developed which would replace the casts and hence prevent the problems such as ulcer, skin irritation and infections caused due to casting for longer period. These splints/braces can as well be used post tenotomy and are designed such that the foot can be fixed at desirable angle in 2 planes by clinicians depending on severity of deformation. These braces can be used for each leg separately to allow their free movement and does not require a connecting bar. Design of this splint can be scaled or can be developed in fixed sizes for various age groups. This model will be put up for clinical trials with the help of NGO, which deals in treating clubfoot and distributing braces.*

KEYWORD: *Clubfoot, Congenital Talipes Equinovarus, Ponseti, Splint, Brace*

BT
BIOTECHNOLOGY (POSTERS PRESENTATION)

Isolation and Characterization of Lectin from *Punica granatum (Pomegranate)*

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ABSTRACT—*Lectins are glycoproteins which recognize carbohydrates, agglutinate the cells and are detected in biological materials by hemagglutinating activity (HA) assay. This study aimed to isolate and characterize the P. granatum testa lectin. The lectin was isolated and extracted from the pomegranate seeds by extraction with phosphate buffered saline (PBS) at pH 7.2. The sugar and the protein content was estimated .The hemagglutination activity was performed on human erythrocytes of blood groups A, B, O, and AB +ve and was found that pomegranate lectin was A and O +ve blood groups specific .The physicochemical characterization of the Pomegranate seed lectin was studied with respect to pH and temperature stability and optimum pH and temperature was found to be'5' and '50ᵒC'. The hemagglutination inhibition assay was performed and pomegranate seed lectin was found to be lactose and arabinose specific. This test shows that Pomegranate lectin has greater affinity towards monosachharides such as Arabinose and Lactose which explains the interactions of lectins with cell surface receptors. This interaction between the lectin and the sugars lead to the immuno modulation. Also the pomegranate lectin showed cytotoxic effect against the MCF- 7 cell line and the IC50 value of 185.4 µg/ml dose-dependent inhibition of growth in MCF-7 cells. The anti mitogenic nature of this lectin may be due to its binding capacity with suitable membrane receptor thus influencing the signal pathway to inhibit mitosis in those cancer cells. The anti mitogenic nature of this lectin needs to be experimented in further studies. Thus the present study may help to contribute in the future day's cancer therapy.*

KEYWORDS: *Lectin, Hemagglutination, Physicochemical Characterization, Heat Labile, Immune-Modulation, Anti-Mitogenic, Dose Dependent Inhibition*

Synthesis of Stable Gold Nanoparticles and Functionalization with Alkaline Phosphatase for a One-Step Nano-based Assay

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ABSTRACT—*Gold nanoparticles (AuNPs) is a topic of growing interest among researchers, especially in the field of bio sensing and the development of nano-bioassays due to their unusual optical properties. The functionalization of metal nanoparticles with enzymes was found to enhance the properties of the enzyme such as activity and thermal stability. In the present study, microwave assisted synthesis of stable AuNPs colloid was performed using citrate as a reducing and stabilizing agent. Functionalization of synthesized AuNPs with alkaline phosphatase (the most commonly used enzyme in immunoassays) was achieved via NHS/EDC chemistry. Preliminary characterization of AuNPs and optimization of synthesis parameters was done with the aid of UV visible spectroscopy. Progress of surface modification of AuNPs and functionalization of alkaline phosphatase (ALP) on AuNP surface was evaluated by monitoring the change in surface plasmon resonance properties of the nanoparticles. The AuNP colloids and the bioconjugates are further to be characterized using TEM, FTIR and XRD analysis. The developed ALP-AuNP bioconjugates can be tagged to antibodies in order to increase the sensitivity and resolution of immunological assays by several folds.*

KEYWORDS: *Gold Nanoparticles, Surface Modification, Alkaline Phosphatase, Immunoassays*

Impact of Extraction Techniques on Antioxidant Potential, Phenolic and Flavonoid Contents of *Mucuna macrocarpa* beans: Response Surface Methodology Optimization

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ABSTRACT—*The antioxidants derived from the natural sources have great medical potential. The present investigation was undertaken to optimize the effective extraction of antioxidants, phenolics and flavonoids from the Mucuna macrocarpa beans. Ultrasound-assisted extraction (UAE) technique and water as an effective solvent were proposed for the response surface methodology (RSM) optimization. The three-level two factors Central Composite design (CCD) was employed to reveal the optimal points of variables. The optimum extraction time (5, 10, 15 min) and ultrasonic power (10, 20, 30 W) were used for the RSM optimization. The extract obtained from UAE and water extraction by RSM were evaluated for the total phenolics content (TPC), total flavonoids content (TFC), DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity, DMPD (N, N-dimethyl-p-phenylenediamine) radical scavenging activity and FRAP (Ferric reducing antioxidant power). ANOVA statistical study showed that time and ultrasonic power had a significant effect on TPC, TFC, DPPH, DMPD and FRAP antioxidant potential. The predicted time 10 min and ultrasonic power 20 W showed maximum extraction of TPC, TFC, DPPH, DMPD and FRAP respectively. The experimental results were in agreement with the predicted response by RSM. The findings of this study implied that UAE was the effective technique and water as a potential solvent for the extraction of TPC, TFC and antioxidant from the Mucuna macrocarpa beans; which leads its application in oxidative stress-related disorder.*

KEYWORDS: *Antioxidants, Mucuna Macrocarpa, Phenolic Compounds, RSM, Ultrasound-Assisted Extraction*

Production and Characterization of Hydrophobic Bacterial Cellulose for Fabrics

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ABSTRACT—*Microbial cellulose is increasingly gaining popularity in the field of biomaterials due to its high crystallinity, purity and its ability to form composites as compared to plant cellulose. Many approaches have been used earlier to impart hydrophobicity to bacterial cellulose. Surface modification done by using hexanoic and dodecanoic acid (Lee it al. 2011) and in situ modifications using silica and titanium nano particles have been some popular approaches. This scientific research aims to view bacterial cellulose as a potential product for apparels and foot wears. Tensile strength and hydrophobicity are two of the desired properties required in fabrics. The working design to induce hydrophobicity in bacterial cellulose was done by providing nano structures on the cellulose surface using silica and stannous nano particles and polydimethylsiloxane and a comparative study was done to see the most effective reagent by water holding capacity test. Furthermore CMC plasticizer was added to increase the tensile strength. The outcome of our research would result in biodegradable, aesthetically pleasing fabrics that significantly influence the future of textile industry in terms of production of ecofriendly clothing.*

KEYWORDS: *Biomaterials, Bacterial Cellulose, Nanoparticles, Hydrophobicity, Textiles*

Rapid and Green Synthesis of Silver Nanoparticles using *Acacia nilotica* Flower Extract for the Selective and Semi Sensitive Detection of Hg^{2+}

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ABSTRACT—*In the present study, a facile and rapid synthesis of colloidal silver nanoparticles (AgNPs) was achieved using Acacia nilotica flower extract as an eco-friendly biomaterial. This biosynthesis process is cost effective, easy and can be a suitable alternative to chemical and physical processes of nanoparticle synthesis. The synthesized AgNPs has been characterized using UV-Vis spectrophotometry and further characterization is to be carried out using Fourier transfer infrared (FTIR) spectrophotometer, Transmission electron microscope (TEM) and X-Ray Diffraction spectroscopy (XRD). The characteristic Surface Plasmon Resonance (SPR) of AgNPs were found to decrease gradually with increase in the concentration of Hg^{2+} with concomitant decolourization of AgNPs colloid. This property of AgNPs in presence of Hg^{2+} has been explored for the development of simple, selective and sensitive tool for the detection of Hg^{2+} owing to its high toxicity to the human beings and aquatic ecosystem. The detection range of the developed sensing tool was found to be in the range of 10^{-3} to 10^{-9} M of Hg^{2+}. In this context, development of simple and portable device for the monitoring of Hg^{2+} is necessary. The present sensing method is simple and easy to miniaturize into a portable system for onsite monitoring of Hg^{2+} in the environmental sample.*

KEYWORDS: *Silver Nanoparticles, Green Synthesis, Acacia Nilotica, Flower Extract, Hg^{2+} Detection*

Comparison of L-Dopa Content in Three Species of Genus *Mucuna* by Different Techniques

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ABSTRACT—*In contemporary medicine, Mucuna remains a genus of interest since its L-DOPA content and use in treatment of Parkinson's disease continues to be evaluated in biochemical research. In the present study, attempts are made to develop suitable method(s) for extraction of L-DOPA from the powdered seeds of 3 species of Mucuna using different solvents and conditions. The seed powder of all plants was subjected to 6 different extraction methods, with different solvent ratios. All the extracts were analyzed using RP- HPLC and were validated according to The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The L-DOPA extraction was best with Methanol Water mixture in a cold maceration technique and overall gives good extraction efficiency in all the three plants giving concentrations of 5.03%, 13.36 % and 16.78% of L-DOPA in Mucunagigantea, Mucunapruriens and Mucunamonosperma, respectively There are not many studies done for optimization of extraction technique for L-DOPA despite an extensive work is reported for isolation, identification and pharmacological activities of L-DOPA from various plant sources. Keeping this in view, present investigation was done to study the extraction efficiency of various extraction methods of L-DOPA content in seed extracts of Mucuna and compare it.*

Synthesis and Characterization of Gold Nanoparticles from the Seed Extract of *Mucunamonosperma*

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ABSTRACT—*Nanotechnology is making an impact in every field of life. Synthesis of nanoparticles from various physical and chemical methods has been reported, having negative impact on environment. The use of plant material not only makes the process eco-friendly but also the abundance makes it more economical. The production of nanoparticles using plant extract is alternative to the conventional methods. This study reports the biological synthesis of gold nanoparticles by the reduction of Chloroauric acid ($HAuCl_4$) by using Mucunamonosperma seed extract. High L-DOPA concentration in Mucunamonosperma seeds fascinated us to utilize it for biosynthesis of gold nanoparticles. Mucunamonosperma seed extract at various % concentration was used to synthesise gold nanoparticles. Analytical tool like FTIR, UV-Visible spectrophotometer and Scanning Electron microscope helped understand the surface chemistry of the gold nanoparticles. The gold nanoparticles synthesised showed spherical shape and FTIR supports the possibilities of L-DOPA coating over it. The results from SEM support the biosynthesis of spherical shaped Gold nanoparticles between 10nm to 50 nm.*

Rapid Synthesis of Silver Nanoparticles using Aqueous Extract of Waste Tea and its Antibacterial Activity

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ABSTRACT—*In the present study, the silver nanoparticles (AgNPs) had been successfully synthesized by using aqueous extract of waste tea powder. The synthesized AgNPs were characterized by UV-Vis spectroscopy and Fourier Transform Infrared (FT-IR) spectroscopy. The AgNPs have absorption maxima at 426 nm. The AgNPs are evaluated for their antibacterial activity against Escherichia coli (Gram-Negative) and Bacillus licheniformis (Gram-Positive bacteria). Antibacterial assay shows that the AgNPs have good inhibition effect on both gram negative and gram positive bacteria. The current study demonstrated the potential use of aqueous extract of waste tea as a novel source of AgNPs synthesis with improved biomedical applications.*

KEYWORDS: *Waste Tea, Silver Nanoparticles, Antibacterial*

Alzheimer's Disease: Metallobiology and Prevention

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ABSTRACT—*Alzheimer's disease (AD), a leading cause of dementia in the elderly, is an irreversible, progressive neurodegenerative disorder clinically characterized by memory loss and cognitive decline that leads to death usually within 7-10 years after diagnosis. Age is the dominant risk factor in Alzheimer's disease, the progressive nature of neurodegeneration suggests an age dependent process that ultimately leads to synaptic dysfunction and neuronal damage in cortical areas of brain essential for memory and higher mental functions. The loss of synaptic functions seems to be the critical factor in cognitive decline. $A\beta$ is a metal binding protein Cu^{2+}, Zn^{2+}, Fe^{2+} promotes $A\beta$ oligomer formation. Cu^{2+} and Fe^{2+} are redox active, generate ROS.*

The drugs currently available to treat Alzheimer's disease address only its symptoms and with limited effectiveness. There is need to develop new therapeutic modalities such as synthetic metal chelators or naturally occurring bioactive molecules to overcome oxidative stress and to inhibit aggregation of amyloid beta ($A\beta$) peptide which plays a pivotal role in the pathogenesis of disease.

KEYWORDS: *Dementia, Cognitive Decline, Redox, Chelators*

Proteomics and Nanoepigenetic Toxicity Analysis of Lung Fibroblast Cells Exposed to Metal Oxide Nanoparticles

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ABSTRACT—*Nanotechnology is growing at an exponential rate undoubtedly having both beneficial and toxicological impact on health and environment. Even if nanoparticles pose health risks, we are exposed to these nanoparticles in day-to-day life by various means such as cosmetics, varnishes, paints, medicines etc. Recent studies suggest that aside from cytotoxic, genotoxic, inflammatory, oxidative stress effects, high concentration of nanoparticles could cause reprogrammation of gene expression and have potential risk of epigenetic modulation.*

In the present study, we report that exposure of lung fibroblast cells (MRC-5) to SiO_2, TiO_2 and ZnO NP at concentrations that lead to nominal cytotoxicity (20% lethality) can have adverse epigenetic effects and modulation of protein expression. The proteomic changes in response to sublethal dose of nanoparticles were visualized by two-dimensional gel and identified by mass spectrometry. Expression of at least 30 proteins was differentially expressed upon exposure to sublethal level of SiO_2, TiO_2 and ZnO nanoparticles. Among these, at least 10 proteins are reported to have a DNA methylation regulated promoters.The most prominent change was DNA hypomethylation induced de-repression of of Vimentin variant 3 which is an important marker in lung cancer. This suggests that the effect of these nanoparticles at sublethal concentration should be studied at the molecular level such as DNA methylation and its corresponding proteome which may contribute to understanding the molecular mechanisms responsible for the toxicity of nano-sized particles.

KEYWORDS: *Metal Oxide Nanoparticles, DNA Methylation, Proteome, Epigenetics*

Statistical Experimental Design for Decolorization of Reactive Red 120 by *Trametes versicolor* (Linnaeus *et fries)* Pilat 1086 FRI 165 (1973)

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ABSTRACT—*Synthetic dyes are extensively used in different industries. Dyes have adverse impacts such as visual effects, chemical oxygen demand, toxicity, mutagenicity and carcinogenicity characteristics. The conventional treatment of dark coloured textile wastewater using chemical coagulation generates large amount of sludge which requires further treatment and disposal. White rot fungi due to extracellular enzyme system are capable to degrade dyes and various xenobiotics. The aim of this study was to check the effectiveness of statistical experimental design for decolorization of reactive red 120 by Trametes versicolor (linnaeus et fries) pilat 1086 fri 165 (1973). Response surface methodology (RSM) was used to study the effect of independent variables. RSM involving a central composite design (CCD) was applied to evaluate the interactive effects of 3 significant factors namely lactose, ferrous sulphate and percent inoculum in different ranges. The results demonstrated the effectiveness of the statistical experimental design and the ability of Trametes versicolor for maximum dye decolorisation (>60%) at the optimum conditions of the significant factors.*

Screening, Isolation, and Antibacterial Activity of Antibiotic Producing Bacteria Obtained from Mangrove Soil Samples

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ABSTRACT—*Antibiotics are antimicrobial compounds produced by living microorganisms. These compounds are used therapeutically and sometimes prophylactic ally in the control of infectious diseases.*

The objective of the present study was to isolate and screen antibiotic producing bacterial from soil samples collected from Mangrove area. Among the 25 bacteria screened, antibacterial activity was exhibited by only one of the isolates (C4). Solid state fermentation and crude extraction were used for the production of antibiotics from isolates. The inhibitory activities of the isolated microorganisms were checked against Staphylococcus aureus, Serratia, Pseudomonas aeruginosa, Escherichia coli, Micrococcus luteus, Bacillus subtilis, Proteus vulgaris and Bacillus subtilis using cross streak method.

Isolation and screening of bacteria from mangrove areas in optimum condition may contribute the discovery of new antibiotics that can fight against antibiotic resistant pathogens. Further identification of bacteria, purification, structural elucidation and characterization of the antibiotics will be carried out.

KEYWORDS: *Isolation of Soil Microbes, Antibiotic, Biochemical Characterization, Antimicrobial Activity*

Detecting Germs in Environment: Options and Limitations

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INTRODUCTION

Imagine a world where the diseases are caught before they catch humans. Diseases lead to the deterioration of the community health. Diseases are majorly spread through air, water and food as well as through vectors such as mosquitoes. It would be really useful if we could develop a system that could detect the presence of virus, bacteria and other biological contaminants in air, water and food and keep a tab on the disease spreading vectors as well so as we can take the needed preventive measures and avoid spread of diseases.

PRIOR WORK AND ITS LIMITATIONS

We surveyed for existing solutions in three parts-whether IoT based systems for disease risk detection exist, whether sensors are available for germs/ biological contaminants detection and whether a home application of this system exists.

- Researchers from Purdue University are working on the long term goal of developing a system for early disease risk detection. As a part of this goal they have developed a sensor which can detect whether a bacteria is alive or dead.[1]

- Engineering students from UMass Lowell have designed "BioBot" which is a low-cost, mobile bio-sensing robot capable of automatically taking air samples in a room and then analysing them to quickly detect any harmful bacteria and inform the required personnel.[2]

- Certain US based researchers have developed "System and method for tracking germ contamination" which routinely monitors germ contamination in areas where human interaction takes places. A germ detection agent is placed either onto such places which gets transferred to the human hands, which can then be scanned under ultraviolet light to know the presence of germs.[3]

Though these current researchers have come up with solutions for detecting the germs (viruses, bacteria, biological contaminants, etc.), our system provides a novel

feature where the connected system is concerned. We discuss our aim of creating an all-round system that not only senses but also uses the concepts of Internet of Things to provide networks and connectivity, puts this information onto the cloud, analyses it and creates conclusions so as to provide better preventive measures as far as healthcare of the society is concerned.

PROPOSED SOLUTION

We aim to discuss the options of using these detection systems (mentioned in literature survey) in our specific application of disease risk detection and discuss other probable detection systems as well. We also aim to go through the limitations of developing such systems. Our aim is to discuss the use internet of things to help us develop this application of disease risk detection system. We aim to discuss different sensors that could be used to detect contaminants within different agents and sources in our houses.

ADVANTAGES AND EFFECTS: APPLICATION AREAS

These systems can be used in places such as hospitals where continuous monitoring of presence of such harmful biological contaminants is a necessity. Such systems could lead to early detection of diseases and better preventive measures thus leading to a healthier society. Schools, colleges, airports, train stations, bus stands, banks, hotels, homes or any other such public places can deploy such a system or at least a part of the system as per the necessity in order to provide a healthier environment to the public.

TECHNOLOGICAL IMPLEMENTATION OF THE IDEA

We will discuss the different sources of germs/ contaminants in our houses and what sensors could be deployed at these places for their detection and continuous monitoring.

Sources of germs/ contaminants and Possible Sensor systems:

- *Water*: We can deploy sensor systems in the source of drinking water supplies in our house. We use water purifiers to sense and kill the germs in our water. UV, UF and Reverse Osmosis based are the most commonly used types of water purifiers in our houses. The UV sensors have been discussed further. The concept of purifying water is very much prevalent, we can say. However a system that would not just kill but also detect the exact number, density of the disease causing agents present in the water, would contribute to analysis of water purity around the city and would help the citizens as well as government in implementing the required policies.

- *Air*: We all know the importance and vitality of drinking purified water. However, we do not realise that the air we constantly inhale also contains thousands of germs. We find the need to discuss methods of purifying air and the sensors that could be deployed for the same. Air conditioners have an air filtering unit. If we could integrate with this system, sensors such as– UV or other virus/bacteria detecting sensors we could monitor the disease causing microbial organisms and germs present in our environment at any point of time. We could make use of the sensors developed by "BioBot"[2] for detecting germs present in the air.

- *Food*: Food contributes as a major vector for transmitting different viruses, bacteria leading to diseases. Though it would be initially hard to employ a scanner that could scan the food each time we eat, we can explore the possibility of fitting germ detecting sensors in places where food is stored. This could include-refrigerators, cupboards, etc. These sensors–probably UV based could scan the food present at regular intervals of time and provide us information regarding presence of disease causing agents.

- *Toiletries*: In this case as well, though it seems fit that we develop a sensor that could scan anything and everything we put in front of it for the presence of disease causing agents, it is difficult for it to be practically realizable. Hence, a realizable solution would be to put up a sensing system that would scan the concerned environment at regular intervals of time. We could also implement the system-" System and method for tracking germ contamination" [3] which can detect germs present on a substance with the help of a powder which gets scanned in the presence of UV light.

- *Shoe Stands, Utensils, Clothes*: To detect the presence of germs/ disease risk posing contaminants a common solution is to put up a sensing system– probably a UV based system where these things are stored i.e. their cupboards.

- *Vectors Carrying Diseases*: Malaria and dengue is spread by mosquitoes, rabies by dogs and plague by rats. There are many other vectors which carry and thus spread diseases and epidemics. It is necessary that we keep a tab on these agents as well to prevent the spread of these diseases. Mosquitoes can be detected in their early stages by using simple techniques such as image processing.

SENSOR TECHNOLOGIES

We further discuss the various sensors required in order to implement our proposed solution.

- The UV technology used today works on the principle that ultraviolet light causes various materials to "fluoresce" or radiate visible light in total darkness which can be used for bacteria and virus detection. Specific wavelengths can be emitted and the spectrum is observed, based on the observations, the germ is detected.

- GFP-The green fluorescent protein can be used as a marker for detection of certain germs/ viruses or agents such as mosquito larvae. These can be deployed through our systems into the required water bodies, etc. and these would implant the gene leading to fluorescence into the organism we wish to detect. The density/ intensity of this fluorescence can be measured to know about the presence of germs/ agents and their density.

- Apart from UV technology which could be used in a wide range of applications, we also need to research on whether we can develop sensors which would make use of thermal imaging or environmental changes like temperature, humidity, etc. to determine the presence of specific viruses, bacteria or other biological contaminants.

- We could use the sensors as used in the "BioBot" system in order to sample air and detect harmful biological contaminants. [2]

- We could use the system as implemented in "System and method for tracking germ contamination" for germ detection through contact.[3]

A common approach to provide a real time monitoring system for detecting presence of germs in any of these possible sources of contaminants seems to be setting up sensor based systems that would scan the required environment after regular intervals of time and upload this data to the cloud. Let us further discuss the use case of this system in a household.

As depicted in Fig.1 sensor systems would be set up in different parts of the house where high germ (virus, bacteria, biological contaminants) density has a higher probability. The cloud would be receiving data from all of these different sensor systems. Each house can have its own cloud portal. On this cloud, the received data would be analysed, a warning would be given and preventive measures would be suggested. A phone application would be developed which would obtain the data from the cloud and provide the information to the user regarding presence of harmful biological contaminants. It could also suggest preventive measures and urge the user to take medical tests. The same data can be analysed to draw patterns and thus analyse if one particular food substance or water or air in a particular area is

contaminated so that the appropriate government department can take preventive measures and deploy appropriate needed policies.
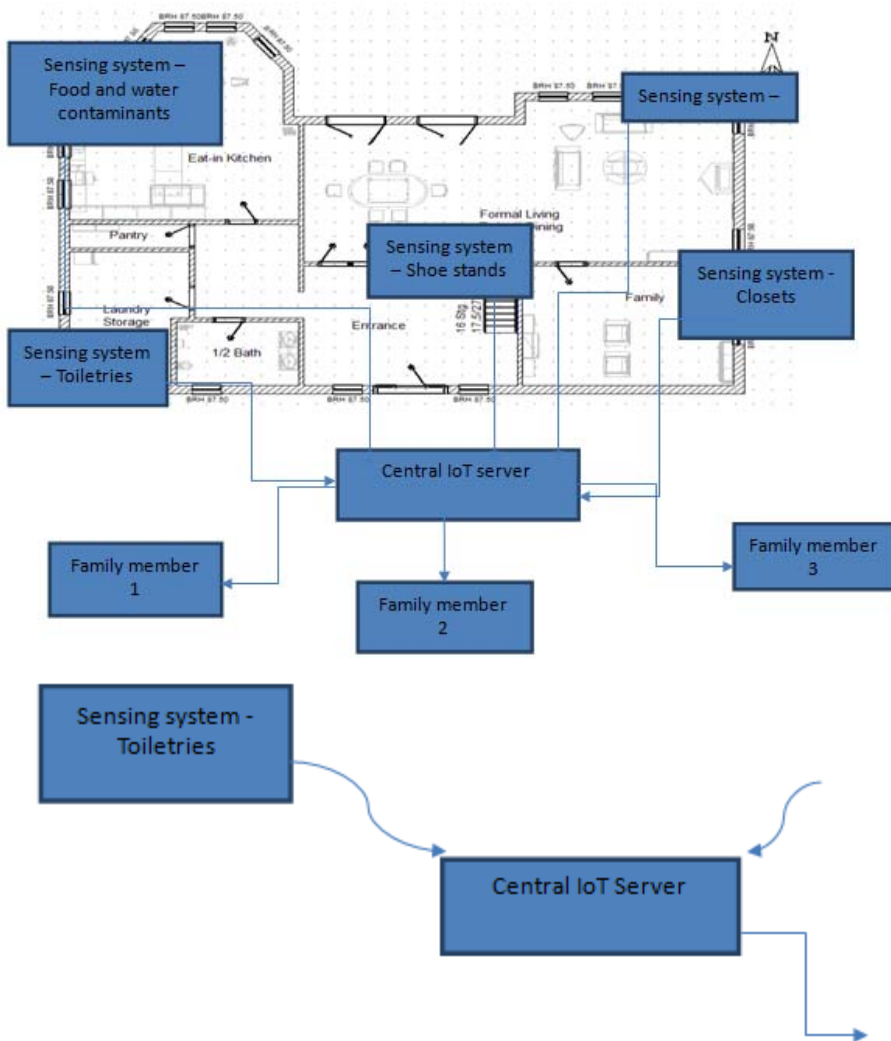
LIMITATIONS

Fig. 1: Block Diagram–Disease Risk Dietection System (Household Applications)

Apart from UV technology there are very few sensor based techniques available for detection of microorganisms. It is practically unrealizable/ difficult to employ detection techniques based on change in environmental factors as the pressure/

temperature, etc. changes caused by the microbial viruses or bacteria are very minute to be sensed. Further research is required as to find out techniques which can detect the presence of these microbial organisms. In our current discussion we proposed that these Ultraviolet based systems be fixed in certain areas where there is a probability of higher germ/ contaminant growth/ density. However it is necessary to research on finding technologies that give further accurate and precise data (e.g. about a particular beverage, food item, or clothes) rather than such average data taken over a wide area. Developing and implementing these systems may turn out to be a costly venture and if a majority of the population cannot benefit from this system it will have a limited positive impact. Hence it is necessary that we research on creating low cost systems and methods for germs/ contaminants detection. In this system we are detecting the presence of germs and contaminants however we are not discussing methods to kill/get rid of these. We focus only on prevention and not on counter-action based on the observed data. We need to research further in order to create systems and technologies that could not just sense but kill the harmful biological contaminants. Implementing this system would require an active participation of the citizens as well as the government body for the betterment of our society.

CONCLUSIONS

We can conclude that the development of an all-round disease risk detection system which would monitor air, water, food, as well as vectors of disease spread such as mosquitoes by sensing presence of germs/ contaminants in different areas of the house having a high probability of housing them and provides the information to a cloud where it is analysed and further action is decided can certainly contribute to early risk detection of diseases and better preventive care facilities. It can lead to the analysis of a large amount of data gathered from all around the city to predict future trends of contaminants/ germs growth and so as for the government as well as the citizens to implement appropriate policies. We hope that developing such a system which detects the disease risk at its most initial state can contribute to developing a healthier, disease-free society

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Comparative Study of Anti-Cancerous Activities of Broccoli Silver and Gold Nanoparticles on Cell Lines

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ABSTRACT—*Plants are our oldest source of medicines. The overall goal of this project is to identify and purify natural chemicals of Broccoli plant and to test their anti-cancerous activity as potential medicines on cell lines. Broccoli plant has been marked as a health promoting food. Sulforaphane is a major component which possess anticancer & antioxidant activities. Broccoli silver nanoparticles & gold nanoparticles were synthesized by biochemical method. Energy Dispersive Spectroscopy (EDS) graph, UV-Vis spectroscopy confirmed that silver and gold nanoparticles were successfully synthesized. Scanning Electron Microscopy (SEM) confirmed the shape and size of silver and gold nanoparticles.*

When compared it is observed that Broccoli silver nanoparticles were more effective than its crude extract & gold nanoparticles on HeLa cell line where as Broccoli gold nanoparticles were more effective than its crude extract & silver nanoparticles on THP-1 cell line.

Identification of plants with compounds active against important human diseases and subsequent characterization of such compounds will lay the foundation for new drug development and lead to novel treatment therapies and better outcomes for patients.

KEYWORDS: *Broccoli, Nanoparticles, Cell Lines*

Antibacterial and Anti-Inflammatory Studies of Dithiocarbamate-In-silico and In-vivo

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ABSTRACT—*Peptidomimetics are compounds whose essential elements (pharmacophore) mimic a natural peptide which retains the ability to interact with the biological target and produce the same biological effect. The present study includes an attempt to mimic the structure of amino acids by linking two amino acids to* **dithiocarbamate** *compound in which one amino acid is protected using protecting groups like Fmoc, Boc and Cbz. Five target proteins were selected viz., 70S ribosome from E.coli (MTCC NO 118) PDB ID: 4V49, Sortase A from Staphylococcus aureus (MTCC NO 121) PDB ID: 1T2W and inflammation causing proteins such as COX1, COX2, and LOX5 protein (PDB ID: 1CQE, 1CX2, 3V99 respectively). Minimization and active site prediction was done to carry out the docking studies. On the basis of docking scores and the number of hydrogen bonds obtained by the molecules, five potent lead amino acid linked dithiocarbamate molecules were selected. They are SIT1, SIT2, SIT3, SIT4 and SIT5. Dynamic study was carried out to confirm the stability of the peptidomimicked structures using Discovery Studio 3.5. The selected lead compounds based on in silico work were synthesised from protected amino acids via mixed anhydride reaction. The synthesized compounds were characterized by ¹H NMR, ¹³C NMR, and HRMS analysis. The characterization results showed that the desired compounds were synthesized with approvable purity and the structural details of compounds were obtained and matched to the in-silico results. The synthesized compounds were evaluated for antibacterial studies against the E.coli and Staphylococcus aureusby using Kirby Bauer agar well diffusion method and In vivo anti-inflammatory studies like carrageenan model, tail flicking and writhing model.. The synthesized potent lead compounds showed better biological activities such as antibacterial and anti inflammatory.*

KEYWORDS: *Peptidomimetics, Molecular Docking, Antibacterial, Anti-inflammatory and Molecular Dynamics*

Tearless Investigations: Bridging the Gap

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Human saliva plays a vital role in maintaining the integrity of oral tissues. Its composition changes during childhood due to maturation of the salivary glands indicating the need of age-matched controls for the clinical use of saliva as a diagnostic tool for diseases. Saliva has been studied extensively as a potential diagnostic tool over the last decade due to its ease and non-invasive accessibility along with its abundance of biomarkers, such as genetic material and proteins. It has long been recognised that saliva serves as a mirror of body's health as it contains proteins that are frequently measured in standard blood tests to monitor health and disease. In the coming future, there are rich possibilities that salivary diagnostics can not only be used as a powerful tool for saving life but also to preserve those, which already have been saved.

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