Unveiling Dysregulated Gene Expression Analysis in Vitiligo and A Computational Approach for Identifying Promising Drug Targets

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Submitted by:

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CANDIDATE'S DECLARATION

I, **Sakshi Rajesh Kumar**, Roll No. **2k21/MSCBIO/37**, student of **M. Sc in Biotechnology**, hereby declare that the project Dissertation titled **"Unveiling Dysregulated Gene Expression Analysis in Vitiligo and A Computational Approach for Identifying Promising Drug Targets"** which is submitted by me to the Department of Biotechnology, Delhi Technical University Delhi in partial fulfilment of the of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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I hereby certify that Project Dissertation titled **"Unveiling Dysregulated Gene Expression Analysis in Vitiligo and A Computational Approach for Identifying Promising Drug Targets"** which is submitted by **Sakshi Rajesh Kumar**, Roll No. **2K21/MSCBIO/37**, Department of Biotechnology, Delhi Technological University in partial fulfilment of the requirement for the award of the degree of Master of Technology, is record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere

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Place: Delhi **SAKSHI RAJESH KUMAR**

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ABSTRACT: -

Vitiligo is a chronic autoimmune disease characterized by the destruction or dysfunction of melanocytes, leading to the emergence of depigmented white patches on various parts of the body. Firstly, focusing on the gene expression of genes implicated in vitiligo and in further study the genes that exhibit overexpression or under expression in this disease, using the Gene Expression Omnibus (GEO) database were observed. To accomplish this, various analytical techniques such as volcano plots, mean-difference plots, and box plots were employed to explore the involvement of these genes in the disease pathology. Based on the dataset analysis, a set of ten up-regulated and ten down-regulated genes were carefully selected for further investigation. In order to stabilize these genes, potential inhibitors, small molecules, or drugs were identified through the drug gene budger. Additionally, a protein known as 6AAH, which acts as a JAK-STAT inhibitor, was chosen after from the Protein Data Bank (PDB) and its structural characteristics were observe using Pymol. Following these preliminary steps, therapeutic intervention using ***Carica papaya***, a medicinal plant, was explored for its potential in vitiligo treatment. Five specific phytochemicals were selected, and evaluate their binding energies with the 6AAH protein through molecular docking using MGL AutoDock. Furthermore, the blood-brain barrier (BBB) permeability of these compounds was assessed using SWISS-ADME to ensure their safety and efficacy. Among the tested phytochemicals, riboflavin demonstrated the most promising results in terms of binding affinity with the 6AAH protein. To optimize the outcomes, Poserlean, another medicinal plant, was selected, and its toxicity, BBB permeability, and interaction with the 6AAH protein were evaluated using MGL AutoDock. To validate and consolidate the findings, extensive simulations were performed to enhance the clarity and comprehensibility of the obtained results

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CHAPTER 01

Introduction

A person's life can be significantly affected by vitiligo, both physically and mentally. The following are some effects of vitiligo on people: Visible depigmented patches have the power to radically change a person's physical appearance. This alteration in skin pigmentation, particularly if it affects prominent parts like the hands, face, or limbs, can cause self-consciousness and a poor perception of one's physique. [1]Vitiligo can have psychological and emotional side effects, including feelings of humiliation, self-consciousness, and poor self-esteem. Because vitiligo may have a negative effect on a person's self-esteem and general well-being, those who have it may also experience anxiety, sadness, social isolation, and a lower quality of life. Social Stigma and Discrimination: People with vitiligo may experience social stigma and discrimination as a result of misunderstandings and societal prejudices.[1–3] Their mental anguish and isolation may increase as a result of unfavourable responses, taunting, or bullying. Relationship Impact: Vitiligo can have an impact on friendships, love relationships, and family dynamics. Due to the fear of being rejected or being judged based only on looks, it may make it difficult to establish new connections or sustain those that already exist. Vitiligo causes depigmented patches of the skin to become more sensitive to sunlight and vulnerable to sunburn.[4,5] Vitiligo sufferers should take extra steps to avoid UV damage and lower their chance of developing skin cancer, such as using sunscreen and protective clothes. Treatment Obstacles: Although there are treatments for vitiligo, managing it can be difficult. Finding a regimen that works for a particular patient might take time and patience, and treatment outcomes vary. Multiple therapies, way of life changes, and frequent follow-up visits with healthcare professionals may all be part of the process.[6]

The primary symptoms of vitiligo are appearance or white patches on skin. Characteristics of patches can vary from person to person. Common symptoms like depigmented patches, symmetrical distribution, progression, loss of colour in hair and eyebrows etc. Diagnosis of vitiligo involve a combination of medical history, physical examination and sometimes additional tests. While there is currently no cure for vitiligo, several treatment options are available to manage the condition and improve appearance of vitiligo. Treatment options may include topical corticosteroids, topical calcineurin inhibitors, Psoralen plus ultraviolet (PUVA) therapy and Narrowband ultraviolet-B (NB-UVB) therapy.

Immune tolerance is compromised, which causes the immune system to mistake melanocytes for foreign substances, which causes them to be destroyed by the immune system in vitiligo. Targeting melanocyte antigens, autoreactive T cells, especially CD8+ T cells, start an immune response against these cells.[7] There is a cytokine imbalance, with pro-inflammatory cytokines on the rise and anti-inflammatory cytokines on the decline. This imbalance helps to activate and kill melanocytes by the immune system together with the overexpression of HLA class I molecules on melanocytes. Uncertainty exists regarding the precise causes of the autoreactive T cell response and the decline in immunological tolerance. To better comprehend these pathways and create tailored therapeutics to control the autoimmune response in vitiligo, more study is required. [8]

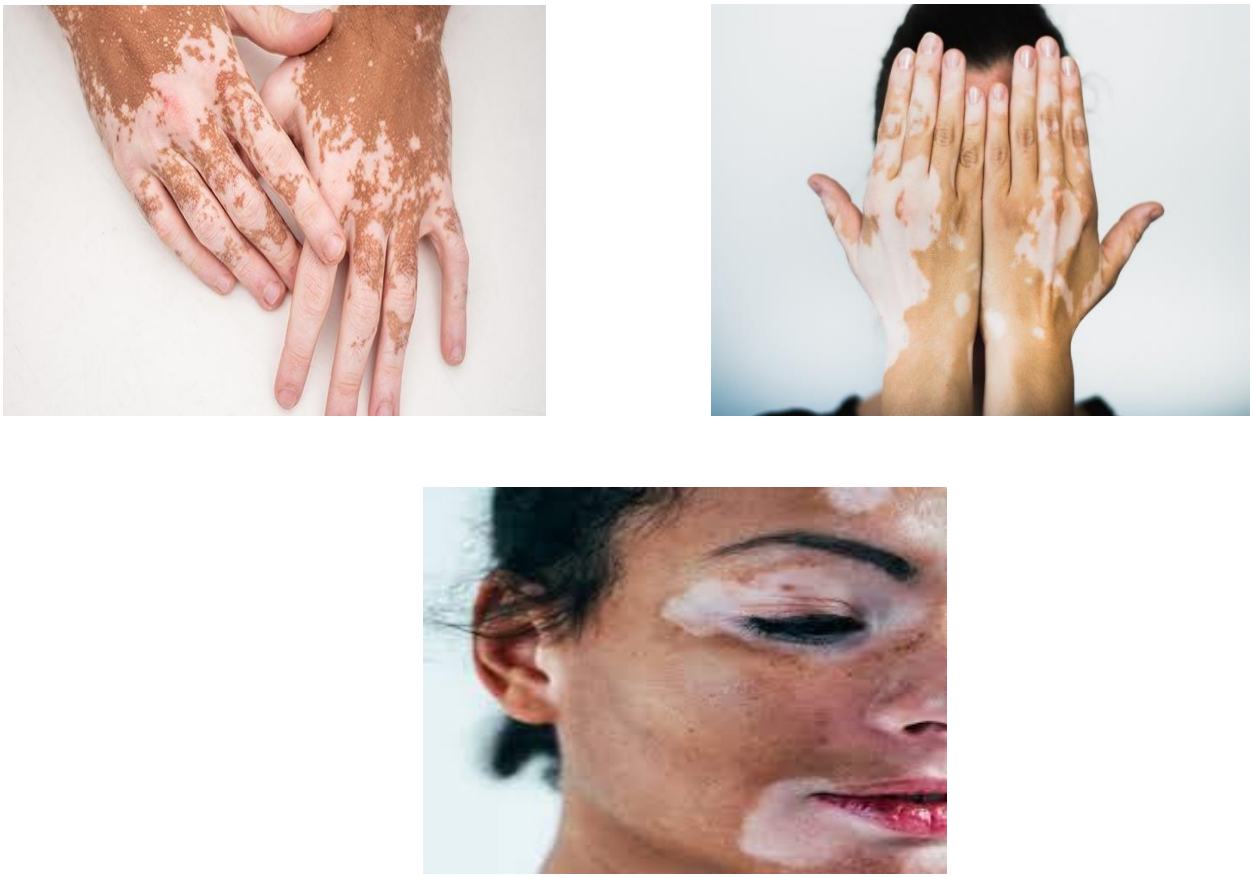

Figure 1. Vitiligo Patches

When someone has vitiligo, their melanocytes are destroyed by the immune system. The tropical fruit papaya is full of vitamins A, C, and E, antioxidants, and enzymes like papain, as well as other minerals. Although papaya has received accolades for its alleged health advantages, there is less evidence to support its use in treating vitiligo, a chronic skin disorder that is characterised by the loss of pigmentation in some places. [9]Due to its high vitamin C content and papain enzyme, some proponents of complementary medicine speculate that papaya may aid in the treatment of vitiligo. Papain is thought to have skin-lightening qualities, while vitamin C is known to be involved in collagen formation and plays a role in maintaining healthy skin. There is presently no solid scientific evidence to support the efficacy of papaya or its components in treating or controlling vitiligo, therefore it's crucial to keep in mind that these claims are primarily anecdotal. It's always better to speak with a dermatologist or healthcare provider if you have vitiligo or any other medical problem so they can offer evidence-based advice and suggest suitable therapies.[10] They will be able to provide you the most recent and trustworthy advice on controlling vitiligo, and they can even make suggestions for additional, scientifically researched and proven effective treatment choices.[11]

Chapter 02

Literary Review

A persistent skin disorder called vitiligo is characterised by a loss of cells that produce colour. Its first recorded occurrences were in ancient Egypt and India, when it was known as "white leprosy" and "Kilas." It was acknowledged as a separate disorder from leprosy in the contemporary age. [12]Better knowledge and treatment choices, such as topical treatments and phototherapy, have been made possible by recent advancements. Although there is no treatment for vitiligo, current research hopes to make life better for individuals who have it.[13]

2.1 Vitiligo Classification

The distribution and pattern of the white patches on the skin are two variables that may be used to classify the different forms of vitiligo. The following are the categorization schemes that are frequently applied to vitiligo:

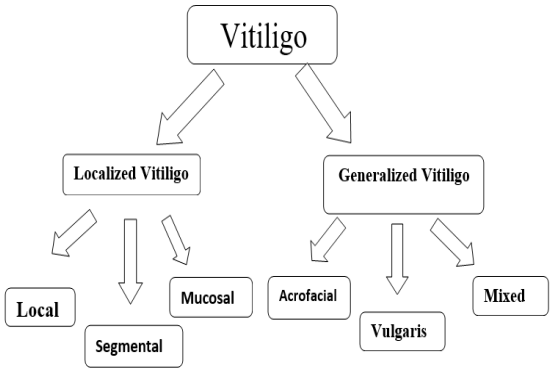

Figure 2. Classifications of vitiligo

i. Segmental Vitiligo: This form of the disease usually only affects one side or body part, such as a particular region of the face, trunk, or limbs. It often starts to appear at a young age and progresses for a short time before stabilising. Segmental vitiligo is less prevalent than other forms and frequently has a quick onset. Non-Segmental Vitiligo (also known as Generalized Vitiligo.

ii. Non- segmental: The most prevalent kind of vitiligo, non-segmental vitiligo, often manifests as bilaterally symmetrical patches. It can strike at any age and affect the face, hands, feet, elbows, knees, and genital regions, among other parts of the body. Non-segmental vitiligo can advance gradually over time and is sometimes linked to other autoimmune diseases.[13]– [15]

There are many subtypes of non-segmental vitiligo:

A single or a few isolated patches appear in a specific area of the body as part of the focal vitiligo subtype.

Acrofacial vitiligo mostly affects the face, particularly the regions around the eyes and lips, as well as the extremities (such as the hands and feet).

c. Mucosal vitiligo: This condition results in the depigmentation of mucous membranes, including the lips, the interior of the mouth, and the genital region.

d. Universal Vitiligo: Universal vitiligo refers to extreme depigmentation encompassing a large section or practically the whole body.

Mixed Vitiligo: This condition is characterised by features of both segmental and non-segmental vitiligo. Segmental and non-segmental patches may both be present in certain circumstances. It's important to keep in mind that vitiligo's categorization might change somewhat based on the sources and research used.[16] The right classification aids dermatologists in establishing the course of treatment, monitoring of the illness, and prognosis for specific patients

2.2 Mechanisms of Vitiligo

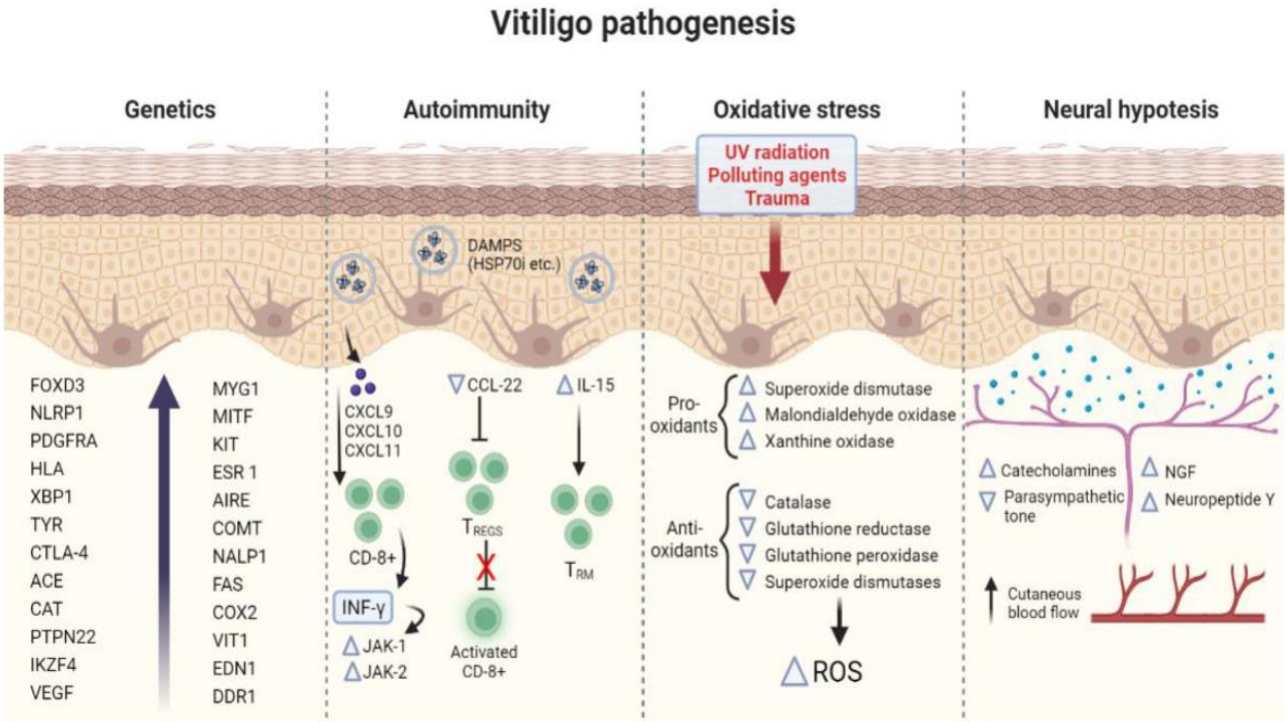

Figure 3. Mechanisms of Vitiligo

1. Genetics

Genetic and environmental variables both have a role in the likelihood of developing vitiligo, which has a multifactorial, polygenic inheritance. 20% of the risk is attributable to environmental variables, whereas 80% is ascribed to genetics. According to family research, 23% of monozygotic twins have concordance, and 20% of patients have a first-degree relative who is afflicted. The heritability of vitiligo is 71% attributed to common genetic variations and 29% to unusual genetic variants[16,17]. 54 vitiligo susceptibility loci have been found by genome-wide association studies (GWAS), the majority of which are connected to immunological control, melanocyte identification, and apoptosis, and some of which are also seen in other autoimmune illnesses. Additionally, using genome-wide linkage research, a number of genes have been linked to vitiligo, including FOXD3, NLRP1, PDGFRA, HLA, and XBP1. Additionally, using genome-wide linkage research, a number of genes have been linked to vitiligo, including FOXD3, NLRP1, PDGFRA, HLA, and XBP1. Additional genes linked to the pathophysiology of vitiligo include MITF, ACE, PTPN22, CAT, and CTLA-4. [18], [19]Recent research suggests a link between vitiligo and VEGF polymorphism, with VEGF being involved in the control of angiogenesis.[20]

2. Autoimmunity

The autoimmune theory is supported by genetic research as the main mechanism causing vitiligo. Approximately 85% of the genes that cause vitiligo are connected to innate, adaptive, and apoptotic immunity.[21].Patients with vitiligo frequently have organ-specific antibodies, and it is commonly accompanied by other autoimmune diseases. Treatments for cancer that involve immune checkpoint inhibitors can also cause vitiligo. Vitiligo pathogenesis is influenced by both innate and adaptive immune responses, with innate immune cells being triggered by stress signals produced by melanocytes and keratinocytes. In vitiligo lesions, CD8+ T lymphocytes are essential for the melanocyte death process. T-cells are recruited to the skin by chemokines and interferon-gamma (IFN-gamma), and dysfunctional regulatory T cells (Tregs) advance illness. Furthermore, increased antibody levels against melanocytes and the persistence of tissue-resident memory T cells (Trm) are seen. In preclinical studies, a number of therapeutic strategies that target immune pathways have shown promise.[8,22–24]

3. Oxidative stress

Melanocyte destruction brought on by oxidative stress plays a major role in the vitiligo development process. The skin's oxidative/antioxidative equilibrium is disturbed by an imbalance between reactive oxygen species (ROS) and antioxidants. Stressors from both within and outside the body cause an excessive amount of ROS to be produced, which destroys melanocytes. Patients with vitiligo are more vulnerable to oxidative stress and have weaker defences. The oxidant-antioxidant balance can be improved by narrowband UVB treatment. Inhibiting the mTOR pathway has potential for treating vitiligo since it contributes to melanocyte dendrite loss. An mTOR inhibitor called rapamycin has been produced in the form of nanoparticles to potentially stop depigmentation.[25]

4. Neural Hypothesis

According to the neural theory, melanocyte death in vitiligo is facilitated by neurochemical mediators secreted by skin nerve endings. This notion is supported by clinical facts, such as the dermatomal distribution of vitiligo patches, their connection to nerve disorders, and the effects of mental stress. Melanocyte destruction is a result of dysregulation of the autonomic nervous system and elevated levels of norepinephrine and neuropeptides. Patients with vitiligo also have high levels of nerve growth factor.[26] Dermal nerves in skin with vitiligo exhibit structural alterations, as seen under an electron microscope.

2.3 Diagnosis

A comprehensive medical history, physical examination, and occasionally further testing are often used to make the diagnosis of vitiligo. The following are the main factors in vitiligo diagnosis: Medical Background: Your symptoms, especially any depigmented patches or changes in skin tone, will be discussed with the doctor, who will also ask whether you have a family history of vitiligo or other autoimmune illnesses.[27] A trigger factor or recent sickness that could be connected to the beginning of vitiligo may also be brought up. Physical Exam: Your skin will be thoroughly examined by the doctor, who will check for any depigmented spots and analyse their location and features. To assist in the assessment of the damaged regions, they could utilise a specialised portable lamp called a Wood's lamp that produces ultraviolet light. [27]Rule out other disorders: In order to rule out other skin disorders, such as tinea versicolor, pityriasis alba, or post-inflammatory hypopigmentation, the doctor may conduct tests or assessments. [19,28]In rare circumstances, skin biopsies may be

performed to aid in the confirmation of the diagnosis and to rule out other disorders. extra Tests: To determine the severity and activity of the condition, extra tests may occasionally be advised. Blood tests to assess thyroid function and look for autoimmune indicators such antinuclear antibodies (ANA) are examples of these examinations. In order to confirm the lack of melanocytes, your doctor may also do a skin biopsy, in which a little piece of skin is extracted and inspected under a microscope.[29]

2.4 Treatment

There is presently no recognised therapy for vitiligo, although there are ways to control the illness and enhance the look of the skin that is afflicted. These remedies consist of: Topical corticosteroids: These lotions or ointments assist in reducing swelling and reducing the immunological response in the afflicted regions. Topical calcineurin inhibitors: To control the immune response and promote repigmentation, medications such tacrolimus or pimecrolimus are applied to the afflicted skin. Topical psoralen plus ultraviolet A (PUVA) therapy comprises using a psoralen cream or ingesting a psoralen medication prior to being exposed to UVA radiation.[30] It encourages the skin's repigmentation. Narrowband ultraviolet B (NB-UVB) therapy: To encourage repigmentation, the afflicted skin is treated to a particular wavelength of UVB radiation. [31]Excimer laser: To promote the formation of melanocytes, this focused laser therapy targets depigmented areas. Depigmentation: Depigmenting the remaining healthy skin may be a possibility in severe cases of vitiligo that cover a significant area of the body. It's vital to remember that therapy efficacy might differ from person to person and that outcomes aren't always predictable. Additionally, controlling vitiligo entails applying sunscreen, limiting exposure to the sun, and getting help from physicians or support groups to deal with the condition's psychological effects. [30], [32]It is advised to speak with a dermatologist if you or someone you know has vitiligo in order to go through the different choices for treatment and choose the most effective way to handle the problem.

Chapter 03

Material and Methodology

3.1. Preparation of Dataset

The NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) provided the publicly available RNA-sequenced dataset utilised in this investigation. The dataset included entire skin samples taken from people who were divided into several groups according to their vitiligo-related skin conditions. These categories comprised samples from healthy people, samples from skin lesions, samples from skin that wasn't impacted by lesions, and samples from the peri-lesional skin.

The dataset was chosen from the GEO database because it has a wide range of freely accessible gene expression data. This gave us access to a large variety of samples that represented different Vitiligo stages and symptoms. A comprehensive depiction of the gene expression patterns across the afflicted regions, including both the epidermal and dermal layers, was made possible by the use of entire skin samples. We were able to document the intricate interplay of genetic and molecular processes that may have contributed to the aetiology of vitiligo using this method.

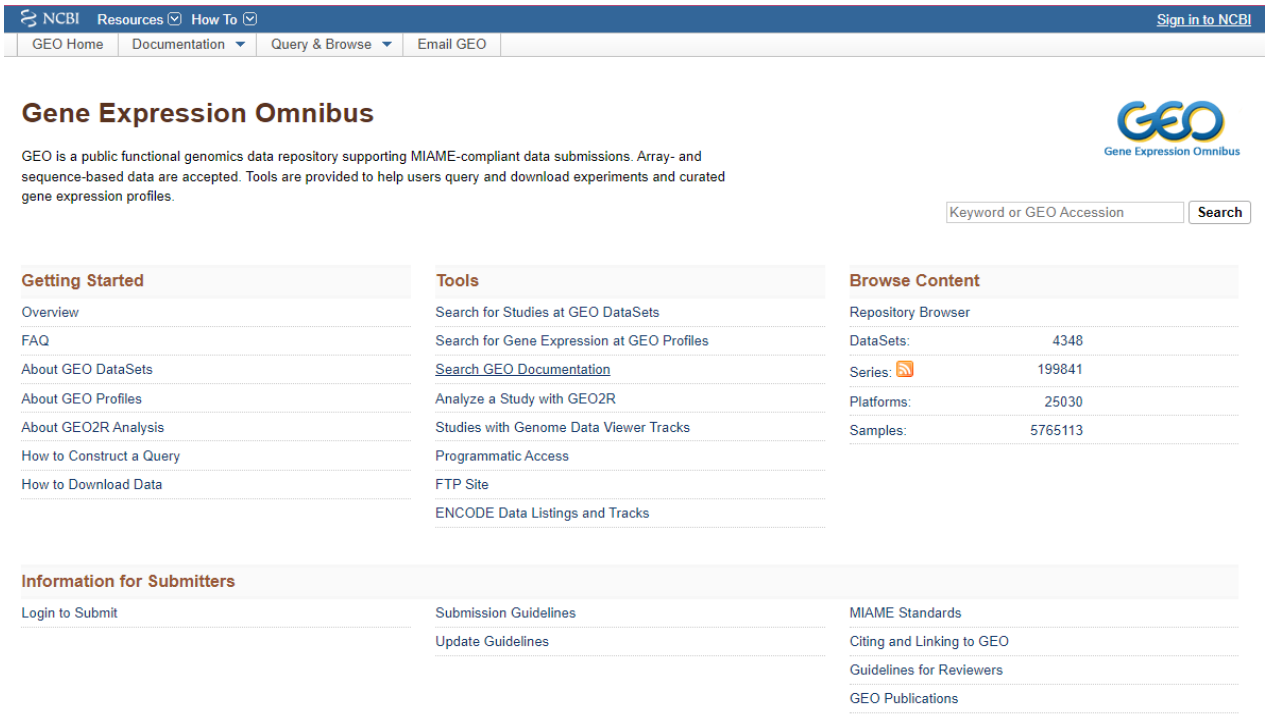

Figure 4. GEO home page (https://www.ncbi.nlm.nih.gov/geo/)

Table 1. Samples Of Vitiligo Affected and Healthy Skin Patients

S.No.	Sample GEO Accession	Sample Title	Group	Tissue
1.	GSM1587709	Healthy [9001_S07_NST]	Control	Whole Skin
2.	GSM1587710	Healthy [9002_S01_NST]	Control	Whole Skin
3.	GSM1587711	Healthy [9003_S02_NST]	Control	Whole Skin
4.	GSM1587712	Healthy [9004_S03_NST]	Control	Whole Skin
5.	GSM1587713	Healthy [9005_S04_NST]	Control	Whole Skin
6.	GSM1587714	Healthy [9006_S05_NST]	Control	Whole Skin
7.	GSM1587715	Healthy [9007_S08_NST]	Control	Whole Skin
8.	GSM1587716	Healthy [9008_S09_NST]	Control	Whole Skin
9.	GSM1587717	Healthy [9009_S010_NST]	Control	Whole Skin
10.	GSM1587718	Healthy [9010_S06_NST]	Control	Whole Skin
11.	GSM1587719	Lesional [9021_S11_LST]	Lesional	Whole Skin
12.	GSM1587720	Lesional [9022_S12_LST]	Lesional	Whole Skin
13.	GSM1587721	Lesional [9023_S13_LST]	Lesional	Whole Skin
14.	GSM1587722	Lesional [9024_S14_LST]	Lesional	Whole Skin
15.	GSM1587723	Lesional [9025_S15_LST]	Lesional	Whole Skin
16.	GSM1587724	Lesional [9026_S16_LST]	Lesional	Whole Skin
17.	GSM1587725	Lesional [9027_S17_LST]	Lesional	Whole Skin
18.	GSM1587726	Lesional [9028_S18_LST]	Lesional	Whole Skin
19.	GSM1587727	Lesional [9029_S19_LST]	Lesional	Whole Skin
20.	GSM1587728	Lesional [9030_S20_LST]	Lesional	Whole Skin
21.	GSM1587729	Non-Lesional[9021_S11_NLST]	Lesional	Whole Skin
22.	GSM1587730	Non-Lesional [9022_S12_NLST]	Lesional	Whole Skin
23.	GSM1587731	Non-Lesional [9023_S13_NLST]	Lesional	Whole Skin
24.	GSM1587732	Non-Lesional [9024_S14_NLST]	Lesional	Whole Skin
25.	GSM1587733	Non-Lesional [9025_S15_NLST]	Lesional	Whole Skin
26.	GSM1587734	Non-Lesional [9026_S16_NLST]	Lesional	Whole Skin
27.	GSM1587735	Non-Lesional [9027_S17_NLST]	Lesional	Whole Skin
28.	GSM1587736	Non-Lesional [9028_S18_NLST]	Lesional	Whole Skin
29.	GSM1587737	Non-Lesional [9029_S19_NLST]	Lesional	Whole Skin
30.	GSM1587738	Non-Lesional [9030_S20_NLST]	Lesional	Whole Skin
31.	GSM1587739	Peri-Lesional [9021_S11_PLST]	Lesional	Whole Skin
32.	GSM1587740	Peri-Lesional [9022_S12_PLST]	Lesional	Whole Skin
33.	GSM1587741	Peri-Lesional [9023_S13_PLST]	Lesional	Whole Skin
34.	GSM1587742	Peri-Lesional [9024_S14_PLST]	Lesional	Whole Skin
35.	GSM1587743	Peri-Lesional [9025_S15_PLST]	Lesional	Whole Skin
36.	GSM1587744	Peri-Lesional [9026_S16_PLST]	Lesional	Whole Skin
37.	GSM1587745	Peri-Lesional [9027_S17_PLST]	Lesional	Whole Skin
38.	GSM1587746	Peri-Lesional [9028_S18_PLST]	Lesional	Whole Skin
39.	GSM1587747	Peri-Lesional [9029_S19_PLST]	Lesional	Whole Skin
40.	GSM1587748	Peri-Lesional [9030_S20_PLST]	Lesional	Whole Skin

3.2 Pre-processing - Dataset Preparation

Analysing the raw RNA-sequencing dataset using the GEO programme to produce a standardised and interactive report. The differentially expressed gene table was then exported. A log fold change (log Fc) and an adjusted p-value were used to further narrow the roughly 2000 genes in this table. Notably, the log Fc filtering criteria involves choosing genes with a log Fc larger than or equal to '2' or less than or equal to -2. In addition, we set the modified p-value's upper limit at 0.05.

3.3 Graphical the observations for comparing the genes between up-regulated and down-regulated

A variety of graphical representations, such as the Volcano graph, Box plot, and Mean Difference plot, provide invaluable insights into the expression patterns of genes related to vitiligo after the analysis and extraction of Healthy and affected gene samples from the Gene Expression Omnibus (GEO) database. The amounts of overexpressed and under expressed genes in this complicated condition may be quickly compared using these graphical tools. These graphs are effective tools for illuminating the differential gene regulation behind vitiligo and promoting a thorough comprehension of the molecular subtleties involved in its aetiology since they visually display variations in gene expression.

3.4 From prepared Dataset selection of up-regulated and down-regulated genes on the basis of graphs and Fc log value

By conducting an in-depth analysis of a comprehensive dataset containing 20,000 genes linked to vitiligo, we unveil a deeper understanding of this perplexing condition. Through the utilization of log fold change values (log Fc), we identify and thoroughly examine the top ten overexpressed genes as well as the top ten under-expressed genes. This meticulous investigation provides illuminating insights into the potential functions and contributions of these genes to the intricate pathogenesis of vitiligo. By unravelling their roles in the disease process, we aim to unravel the underlying mechanisms that drive vitiligo, opening doors to innovative therapeutic strategies and improved patient care.

3.5 Using Drug gene budger for stability of dysregulations of genes

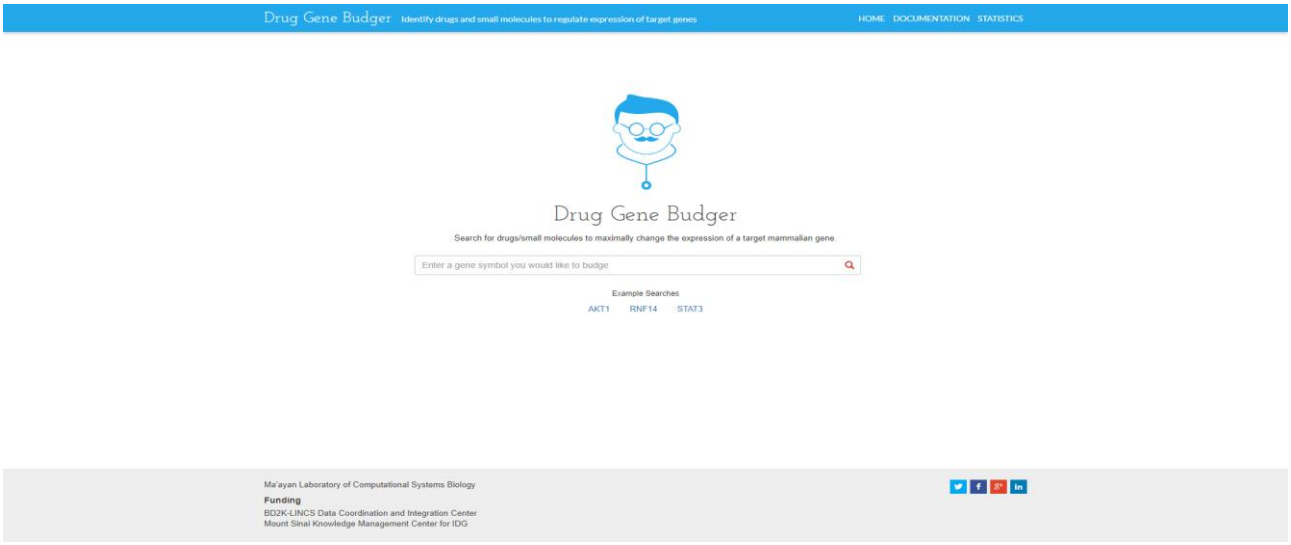

Figure 5. Drug Gene Budger https://maayanlab.cloud/DGB/

The system produced results in accordance with the under- and overexpressed genes entered into the space supplied. Inhibitors or tiny compounds that target under-expressed genes were chosen to stabilise a gene's function if it was discovered to be overexpressed. In contrast, inhibitors and small compounds that target overexpressed genes were chosen when a gene was under expressed. Then, on the following page, three alternatives were displayed: L1000, CREEDS, and CMAP. The L1000 dataset included a number of metrics, including cell lines, time dosage, p-value, log2fold change, and specificity, which offered important insights into the changes in gene expression brought on by various toxins, illnesses, or genetic mutations. The L1000 dataset has transformed systems biology and drug development, made it possible to identify therapeutic targets, and permitted cutting-edge methods for personalised medicine. On the other side, observations in CREEDS focused on the examination of cis-regulatory elements (CREs) in evolutionary genomics and included GEO ID, PUBCHEM ID, and DRUGBANK ID. The vast collection of gene expression profiles from human cells exposed to various small molecules provided by CMAP (Connectivity Map) serves as a comprehensive resource in pharmacogenomics and drug discovery, assisting in the investigation of molecular links between medications, illnesses, and biological processes.

3.6 Using *Carica papaya* as a medicinal plant, researchers are discovering a treatment for vitiligo via silico method.

3.6.1 Integration of Protein 6AAH-Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6- Dimethyl- 7-octene-2,3,6-triol)

The RCSB Protein Data Bank (PDB) provided the protein 6AAH in the FASTA format, along with its ligands myristic acid, heptadecanoic acid, riboflavin, propanol, and 2,6-dimethyl-7-octene-2,3,6-triol taken from pubchem. The binding interactions were then evaluated using the online programme PLIP (Protein - Ligand Interaction Profiler). Surprisingly, as shown by the residual values at locations 879A, 881B, 889B, 101B, and 959B, respectively, all the ligands successfully bound to the protein. The recognised amino acids for these interactions in the given sequence are ARG, LEU, VAL, LEU, and LEU. The protein 6AAH and several ligands were extracted in FASTA format using the RCSB PDB as a reference. The PLIP web-tool subsequently made it possible to analyse the interactions between proteins and ligands in great detail. Notably, the residual results indicated that all the ligands successfully bound to the protein. ARG, LEU, VAL, LEU, and LEU in the provided sequence were found to be the particular amino acids implicated in these interactions.

3.6.2 Procedure for docking proteins or ligands

The structure was cleared of all heteroatoms (atoms other than carbon and hydrogen), polar hydrogen atoms, and water molecules. KOLLMANN charges were given to the ligand and both receptors. The auto-docking software's preferred file format, PDBQT, was used to convert the agonist. For later usage in the auto-docking procedure, the PDBQT file for the agonist and the PDB file for the receptor were both preserved. Using the web application Open Babel, the agonist was transformed into a PDBQT file.

3.6.3 Used the MGL Auto dock tool to bind proteins and ligands.

Software services are offered for the molecular docking of proteins and ligand. The PDB files for the target protein and the ligand were given to the web server in order to perform docking. A grid map was generated as part of the docking procedure to direct the docking computations. The auto-grid file was first started in order to set up the grid. The auto-docking procedure was started after the grid was prepared to carry out the actual docking computations.

3.6.4 Performs a structural analysis of the docked protein-drug combination using the programme Bio via.

To look into the structural interactions between the protein and ligand, the AutoDock results were downloaded and examined using BioVia Discovery Studio software. BioVia Discovery Studio

received the protein-ligand complex and produced a 2D picture to depict the interactions. This picture made it possible to examine the interaction between the ligand and the receptor and made it easier to pinpoint the precise amino acids involved in the binding procedure. This research aided in the knowledge of the amino acid level interactions and binding process between the ligand and receptor.

3.6.7 SWISS ADME analysis of the ligand's pharmacodynamics.

Absorption, Dispersion, Metabolic Activity, and Excretion, or ADME, is the acronym for a group of factors crucial to the research of ligands. These factors are investigated by looking at the ligand's canonical smiles to evaluate several characteristics such water solubility, pharmacokinetics, physicochemical qualities, and lipophilicity. These studies offer important insights on the potency and efficacy of the medication. Drug molecules may be assessed using these criteria using an online programme called SWISS ADME (SwissADME). The tool evaluates the qualities of the medication by entering its canonical smiles. The instrument can also reveal if a medicine can pass through the blood-brain barrier or not. This is shown by a cooked egg, where the presence of the medication in the yolk (BBB+) implies that it can penetrate the blood-brain barrier, but the presence of the drug in the white (BBB-) indicates that it may have trouble doing so.

Chapter 04

Results

4.1 Graphical representation

1. Volcano Plot

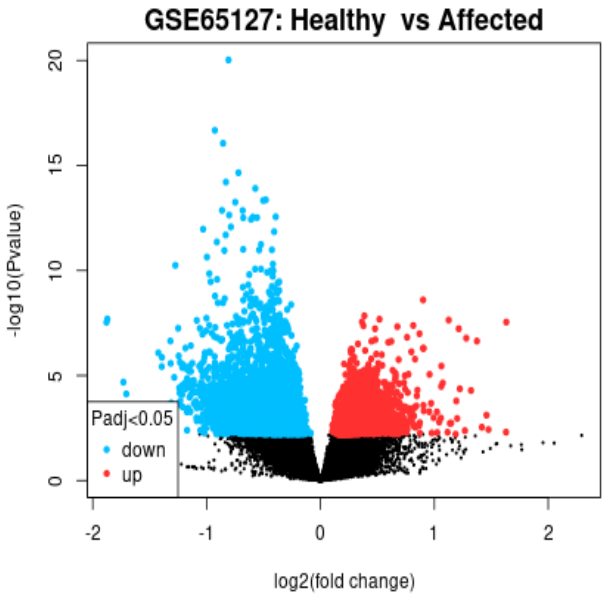

Figure 6. Volcano Plot

Significant alterations in immunological responses, melanocyte function, and inflammation are revealed by a volcano plot analysis of the gene expression associated with vitiligo. Downregulated genes have an impact on pigmentation and melanocyte formation, whereas upregulated genes lead to depigmentation. These discoveries shed light on the molecular underpinnings of vitiligo and suggest new treatment targets. To improve therapy choices and comprehend the complexity of vitiligo, more research, functional analysis, and confirmation of differentially expressed genes are required.

2. Mean-Difference plot

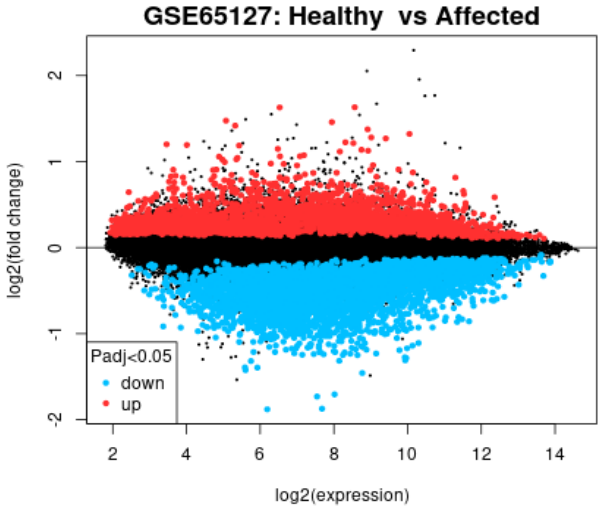

Figure 7. Mean-Difference Plot

Gene expression discrepancies between healthy people and vitiligo sufferers may be shown on a mean difference plot. While negative values suggest downregulated genes, positive values show upregulated genes. This diagram clarifies notable expression discrepancies, the effect of vitiligo on gene expression, and possible underlying processes. Analysis using statistics is necessary for trustworthy interpretation

3. Box - Plot

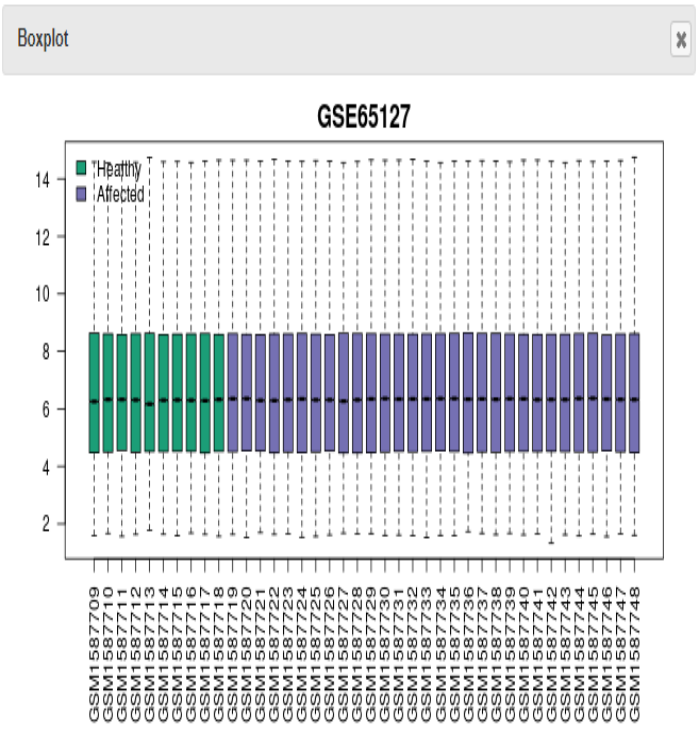

Figure 8. Box-Plot

The distribution of gene expression levels between healthy and vitiligo-affected people is shown in a box plot. Significant differences between the two groups are shown by evaluating the central tendency, spread, and variability of gene expression. Genes that are consistently up- or down-regulated can be found. Box plots also draw attention to possible outliers that show distinctive patterns of gene expression. They offer a succinct summary that makes it easier to see patterns, trends, and possible vitiligo biomarkers. A correct interpretation of observed differences is ensured through statistical analysis.

4.2 About Selected Genes

Table 2. Up-regulated Genes involve in vitiligo

S.No.	GENES	Symbols	p-value	Log Fc
1.	Dopachrome Tautomerase	DCT	7.13E-0.3	2.293847
2	Melan-A	MLANA	1.65E-02	2.052757
3	Tyrosinase related protein 1	TYRP1	3.38E-02	1.76799
4	Transient receptor potential cation channel subfamily M member 1	TRPM1	4.94E-03	1.629243
5	Calpain 3	CAPN3	5.68E-02	0.572567
6	Retinol binding protein 4	RBP4	2.53E-02	0.976543
7	C-C motif chemokine ligand 20	CCL20	2.21E-02	0.940403
8	SRY-box 10	SOX10	6.37E-02	0.468553

9	Insulin like growth factor 1 receptor	IGF1R	6.13E-03	0.468323
10	Carboxypeptidase, vitellogenin like	CPVL	4.19E-01	0.098967

Role of selected genes in vitiligo (Upregulated)

1. **DCT** - Vitiligo growth is dependent on DCT, an essential enzyme in the synthesis of melanin. Depigmentation is caused by impaired melanin production caused by decreased DCT expression.[33] For the purpose of creating pigmentation restoration therapies that may involve boosting DCT expression or enzymatic activity, it is vital to comprehend how DCT works. [34]
2. **MLANA** - The expression of MLANA, a crucial protein for the formation of melanosomes and the synthesis of melanin, is downregulated in vitiligo. It is essential to comprehend MLANA's role in order to create treatments that restore pigmentation.[33]
3. **TYRP1** - In vitiligo, TYRP1 is essential for melanin production. Depigmentation and poor pigment production are the effects of its diminished expression.[35] Enhancing TYRP1 expression or activity may be a way to help vitiligo patients regain their pigment.
4. **TRPM1** - TRPM1, a crucial protein in vitiligo, controls calcium channels and melanin. Loss of pigmentation results from disruptions in calcium regulation and melanin formation caused by reduced TRPM1 expression in depigmented skin.[36]
5. **CAPN3**- A protein called calpain 3, or CAPN3, is involved in the maintenance and repair of muscles. [37]The expression of CAPN3 is changed in vitiligo patients' skin. Its specific function in vitiligo is unclear, however inflammation and immunological responses may play a role.
6. **RBP4**- Retinol-binding protein RBP4 is a component in vitamin A transportation. A possible involvement in melanocyte activity and pigmentation is suggested by altered RBP4 expression in the skin in vitiligo.[38]
7. **CCL20**- The immune-mediated component of vitiligo is influenced by CCL20, an immune-related chemokine. Dysregulation of CCL20 draws immune cells, causes vitiligo lesions to become inflamed, and may harm melanocytes.[39] Understanding the function of CCL20 aids in the understanding of immune dysregulation and promotes the creation of tailored vitiligo treatments.
8. **SOX10** - A transcription factor called SOX10 controls the growth and operation of melanocytes in vitiligo. Loss of melanocytes and depigmentation may result from SOX10

expression dysregulation. [40]The involvement of SOX10 can influence targeted re-pigmentation treatments.

9. **IGF1R** - IGF1R, the insulin-like growth factor 1 receptor, plays a crucial role in vitiligo. It is involved in melanocyte function, survival, and immune regulation. Altered IGF1R expression in vitiligo contributes to melanocyte destruction and the loss of pigmentation. [41]Targeting IGF1R signalling may hold potential for re-pigmentation in vitiligo
10. **CPVL** - CPVL (carboxypeptidase, vitellogenin-like) is a protein implicated in vitiligo. It plays a crucial role in melanin synthesis and regulation. Altered expression of CPVL is observed in vitiligo-affected skin, suggesting its involvement in the disease. [42]–[44]CPVL is involved in processing proopiomelanocortin (POMC), which influences melanocyte function and melanin production. Dysregulation of CPVL may disrupt these processes and contribute to depigmentation in vitiligo. [45]

Table 3. Down- Regulated Genes involve in vitiligo

S.No.	GENES	Symbols	p-value	Log Fc
1.	Signal transducer and activator of transcription 1	STAT1	2.00E-01	-0.1555
2	Aryl hydrocarbon receptor nuclear translocator like	ARNTL	2.04E-08	-1.87168
3	Forkhead box P3	FOXP3	9.64E-01	-0.00314
4	X inactive specific transcript (non-protein coding)	XIST	7.25E-02	-1.48677
5	Cyclin dependent kinase 6	CDK6	2.78E-05	-1.10896
6	Interferon regulatory factor 6	IRF6	3.48E-04	-1.08555
7	C-C motif chemokine ligand 18	CCL18	6.28E-03	-1.06106
8	C-C motif chemokine ligand 10	CXCL10	4.55E-01	-0.51156
9	RAR related orphan receptor A	RORA	7.89E-04	-1.4576
10	Kielin/chordin-like protein	KCP	9.35E-01	-0.00451

Role of downregulated genes involves in vitiligo

1. **STAT1**- STAT1 plays an important role in vitiligo. It is a transcription factor involved in regulating immune responses. In vitiligo, STAT1 is upregulated, leading to increased inflammation and immune activation. This can contribute to the destruction of melanocytes and loss of pigmentation. [46]
2. **ARNTL** - ARNTL, a transcription factor involved in circadian rhythm and immune responses, plays an important role in vitiligo. Its decreased expression in vitiligo lesions disrupts melanocyte function and contributes to depigmentation. [47]ARNTL also regulates genes related to oxidative stress and inflammation.
3. **FOXP3** - FOXP3, a transcription factor associated with regulatory T cells, is involved in immune regulation. In vitiligo, altered FOXP3 expression and impaired Treg function may contribute to the autoimmune response against melanocytes.[36], [48] Understanding FOXP3's role in vitiligo sheds light on immune dysregulation and potential therapeutic targets for restoring immune balance.
4. **XIST** - XIST, or X-inactive-specific transcript, is a long non-coding RNA that plays a critical role in X chromosome inactivation. In the context of vitiligo, the involvement of XIST is not well-established. Vitiligo primarily affects the skin, and it is not typically associated with X-linked inheritance patterns. [49]Therefore, the direct role of XIST in vitiligo is unclear, and there is limited research on its specific involvement in the disease. Further studies are needed to investigate any potential connections between XIST and vitiligo and to understand the underlying mechanisms, if any, of XIST in relation to the pathogenesis of vitiligo.
5. **CDK6** - CDK6, or cyclin-dependent kinase 6, is a protein involved in cell cycle regulation and proliferation. Its role in vitiligo is still under investigation. Studies suggest that CDK6 may be dysregulated in vitiligo, potentially contributing to the abnormal proliferation or survival of melanocytes.[50] Altered expression levels of CDK6 have been observed in vitiligo-affected skin. Further research is needed to understand the exact mechanisms and therapeutic implications of CDK6 in vitiligo.
6. **IRF-6** -IRF6, or interferon regulatory factor 6, is involved in vitiligo and plays a role in immune responses, inflammation, and melanocyte function.[51] Its dysregulated expression in vitiligo-affected skin suggests its involvement in autoimmune processes and immune dysregulation.

7. **CCL18** - CCL18 is involved in vitiligo and is associated with immune responses and inflammation. Its increased expression in vitiligo skin suggests its role in the inflammatory processes of the disease. [13]CCL18 may attract immune cells and influence the progression of vitiligo, but more research is needed to understand its exact involvement.
8. **CXCL10**- CXCL10 is involved in vitiligo and contributes to immune responses and inflammation. It is upregulated in vitiligo-affected skin and attracts immune cells to the inflamed areas. CXCL10 may play a role in the immune-mediated destruction of melanocytes.[13], [14], [52]
9. **RORA** - RORA, or Retinoic Acid Receptor-Related Orphan Receptor Alpha, is involved in vitiligo and its downregulation is associated with the dysregulation of immune responses and melanocyte function[53]

10. **KCP** -KCP (Kinelin cysteine rich BMP) regulator is a protein that can bind and inhibit TGFβ1. Role of TGFβ1 cell regeneration.[53]

4.3 List of Drug/small molecule which help to target the genes which are dysregulated

Table 4. Targeted Gene of interest showing gene expression and the drugs/small molecules targeting them

Genes	Gene Expression	Drugs/Small molecule
DCT	Up regulate	Doxorubicin, Pioglitazone
MLANA	Up regulate	Doxorubicin, Pioglitazone, Imatinib and Celecoxib
TYRP1	Up regulate	Carboplatin, Doxorubicin, Interferon beta-1a
TRPM1	Up regulate	Doxorubicin, Pioglitazone
CAPN3	Up regulate	Motexafin gadolinium (4 h), Etanercept, Doxycycline
RBP4	Up regulate	Tretinoin, Pioglitazone
CCL20	Up regulate	Vemurafenib, Metformin, Doxorubicin, Etanercept, and Plx4032
SOX10	Up regulate	Doxorubicin, Nicotine
IGF1R	Up regulate	Cisplatin, Tretinoin, Vemurafenib
CPVL	Upregulate	Doxorubicin, Nitric oxide, Diclofenac, Cytarabine
STAT1	Down-regulate	Doxorubicin, Tretinoin, Imatinib
ARNTL	Down- regulate	Doxorubicin, Ribavirin
FOXP3	Down- regulate	Doxorubicin, Tretinoin, Imatinib
XIST	Down- regulate	Dasatinib, Imatinib, Nilotinib
CDK6	Down- regulate	Doxorubicin, Cisplatin, Nitric oxide
IRF6	Down- regulate	Resveratrol, Bicalutamide, Diclofenac
CCL18	Down- regulate	Doxorubicin, Bicalutamide, Plx4032
CXCL10	Down- regulate	Doxorubicin, Tretinoin, Ribavirin
RORA	Down- regulate	Doxorubicin, Dasatinib, Diclofenac
KCP	Down- regulate	Tretinoin, Bicalutamide

Table 4 presents a list of selected up-regulated and down-regulated genes in vitiligo, along with their corresponding drug targets. The information regarding these drug targets has been obtained from the Drug Gene Budger, which is a comprehensive database that associates drugs with specific target genes.

The table highlights the potential therapeutic relevance of these genes in the context of vitiligo. By analysing the gene expression changes in vitiligo and identifying corresponding drug targets, researchers can explore the possibility of repurposing existing drugs or developing new therapies to modulate these targets and potentially alleviate the symptoms of vitiligo.

The up-regulated genes listed in the table may represent attractive drug targets for vitiligo treatment. By targeting these genes with specific drugs, it may be possible to modulate their activity and restore normal pigmentation in the affected areas. The down-regulated genes, on the other hand, may suggest potential targets for interventions aimed at addressing the underlying molecular mechanisms associated with vitiligo.

It is important to note that while the presence of drug targets associated with these genes is promising, further research and validation are required to determine the efficacy and safety of specific drugs in treating vitiligo.[54], [55] The identification of drug targets based on gene expression changes is an essential step in the drug discovery process, but it does not guarantee immediate therapeutic success.

Nevertheless, the information provided in Table 4 serves as a valuable resource for researchers and clinicians interested in exploring targeted therapeutic approaches for vitiligo. It highlights the potential for personalized treatments based on individual gene expression profiles and opens avenues for further investigation into the molecular mechanisms underlying vitiligo pathogenesis and potential drug interventions.[56]

4.4. In-silico method results

4.4.1 Ligand (Myristic acid, heptadecanoic acid, riboflavin, propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) and 6AAH interaction

Numerous ligands, including myristic acid, heptadecanoic acid, riboflavin, propanol, and 2,6-Dimethyl-7-octene-2,3,6-triol, have been reported to interact significantly with protein 6AAH. The effective binding of these ligands to the protein has been observed, and the residual values of their binding to the protein are 879A, 881B, 889B, 101B, and 959B, respectively. Myristic acid binds to

the protein through an arginine residue, heptadecanoic acid binds through a leucine residue, riboflavin binds through a valine residue, propanol binds through yet another leucine residue, and 2,6-Dimethyl-7-octene-2,3,6-triol binds through a leucine residue. Furthermore, for these ligand-protein interactions, the hydrophobic interaction distances are as follows: 2.93, 3.83, 4.00, 3.65, and 2.37, respectively**.**

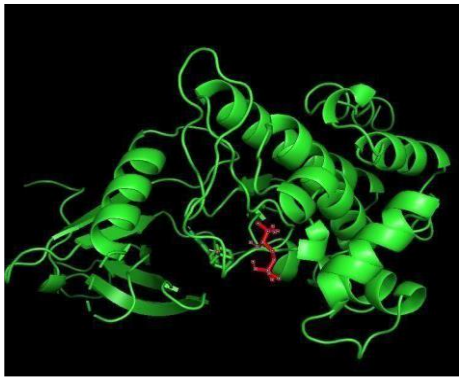

Figure 9. Interaction of 6AAH and 2,6-Dimethyl-7-octene-2,3,6- triol

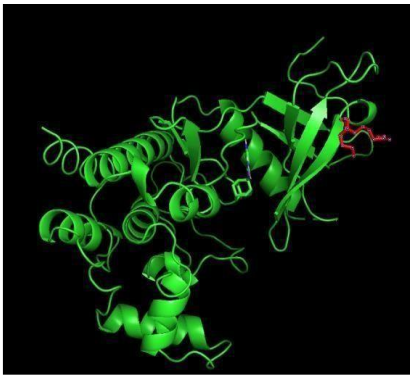

Figure 10. Interaction of 6AAH and Heptadecanoic acid

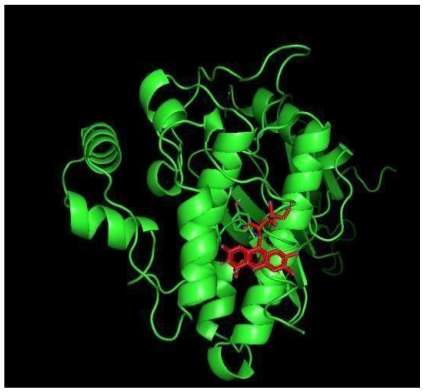

Figure 11. Interaction of 6AAH and Riboflavin

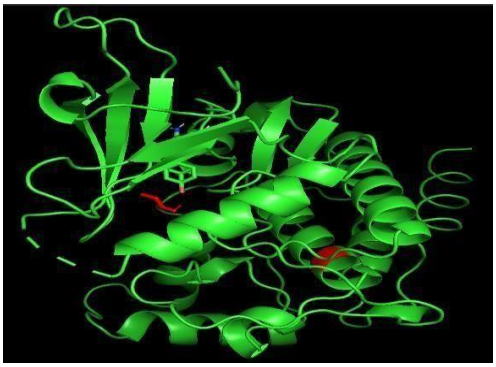

Figure 12. Interaction of 6AAH and Propanol

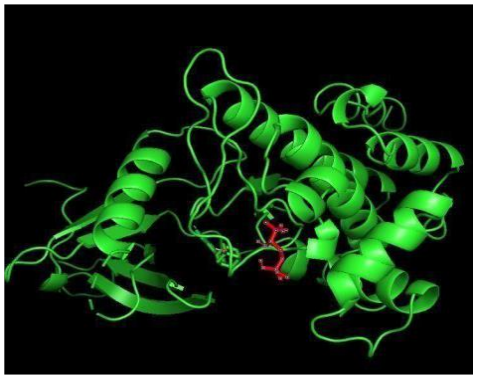

Figure 13. Interaction Of 6AAH and Myristic acid

4.4.2 Ranking of ligands according to interaction via Swiss ADME

The results obtained from the docking performed using AutoDock were highly positive, indicating a strong interaction between the 6AAH protein and the ligands: Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, and 2,6-Dimethyl-7-octene-2,3,6-triol. The docking scores revealed that these ligands efficiently bind to the 6AAH protein complex. The binding energy values were calculated as -4.00 kcal/mol, -2.4 kcal/mol, -6.6 kcal/mol, -3.6 kcal/mol, and -4.00 kcal/mol, respectively. Additionally, the Cluster RMSD value was found to be 0, indicating a high level of similarity among the docking poses.The docking results were analysed to gain insights into the interaction between the molecules. The structure representation displayed the 6AAH protein as a helical structure, colored green and consisting of two chains: A and B. The ligands were depicted as spheroids connected to the protein structure by linear ribbons, illustrating their spatial arrangement. This visualization allowed for an understanding of how the ligands interacted with the receptor site on the target protein.

Table 5. Ranking of Ligands according to interactions

S.No.	Ranks	Ligands
1	Ist	Riboflavin
2	IInd	Myristic acid
3	IIIrd	2,6-Dimethyl-7-octene-2,3,6-triol
4	IVth	Heptadecanoic acid

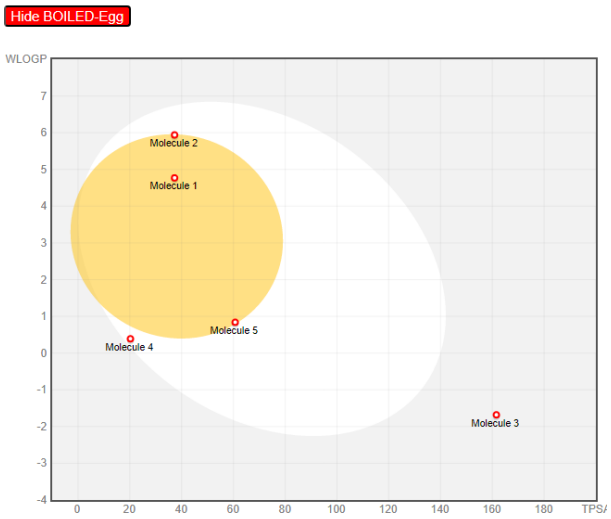

Figure 10. BBB (Blood brain barrier) crossing identified by the help of Boiled egg

4.4.3 Analysation of Ligand through ADME

The skin permeation values, also known as log Kp, were observed to be low in the study. The specific values recorded were -3.35 cm/sec, -2.49 cm/sec, -9.63 cm/sec, -6.49 cm/sec, and -7.14 cm/sec. Lipinski's rule of five was applied to assess the drug's properties, and it was found to meet the required criteria. Additionally, the drug demonstrated proven water solubility based on the log values from ESOL, Ali, and SILICOS-IT categories, which were -4.31, -5.37, -1.31, -0.30, -0.95; -6.67, -8.31, -1.43, -0.24, -1.28; -4.51, 5.71, -2.62, -0.33, -0.72, respectively.

Furthermore, the drug's pharma kinetics score was calculated to be 6.11, 7.69, -1.46, 0.25, and 0.44, providing insights into its behaviour within the body. To determine the drug's lipophilicity, the cast log P o/w (XLOGP3) values were utilized. In summary, the study examined various parameters to evaluate the drug's characteristics, including skin permeation, Lipinski's rule, water solubility, pharma kinetics score, and lipophilicity.

Chapter 5

Discussion

Vitiligo is a significant global issue that profoundly affects the quality of life for those living with the condition. Characterized by the loss of melanocytes, the cells responsible for producing melanin, effective treatment options are crucial in addressing this dermatological disorder. Several factors, including autoimmune disorders, genetics, and potential environmental stressors, have been proposed as possible causes of vitiligo. In this study, our aimed to gain insights into vitiligo by analysing 40 samples. These samples were divided into two groups: Control and Disease, consisting of equal numbers of participants. To identify gene expression differences between the control and vitiligo samples, the researchers employed GEO2R, a widely used tool for comparing gene expression data. Using log fold change (log Fc) values, genes were categorized as upregulated or downregulated. A positive log Fc value indicated upregulation, while a negative value indicated downregulation. Upon analysing the gene expression profiles, the researchers generated various graphs to visualize their findings. The volcano plot provided a comprehensive overview, illustrating the comparison between upregulated and downregulated genes. This plot enabled the researchers to identify genes exhibiting significant changes in expression levels. Additionally, box plots were employed to examine the distribution of gene expression data, ensuring a comprehensive understanding of the dataset's characteristics. To address the management and treatment of vitiligo, researchers turned to the Drug Gene Budger, a comprehensive database linking drugs to specific target genes. By exploring this resource, they identified potential drug candidates or small molecules that could be utilized to target specific genes of interest. The rationale behind this approach is that by modulating the activity of these target genes, it may be possible to stabilize their functioning and mitigate the effects of vitiligo. By leveraging the identified drugs or small molecules targeting the genes of interest, there is potential for significant advancements in the management and treatment of vitiligo. These therapeutic interventions hold promise for improving the stability and functioning of melanocytes, thereby alleviating the symptoms and impact of the disease. However, it is crucial to note that further research and validation are necessary to ascertain the efficacy, safety, and specific mechanisms of action of these potential treatments.

Vitiligo presents a significant challenge to patients worldwide, necessitating effective treatment options. Through the analysis of gene expression profiles and the utilization of advanced analytical tools, researchers have gained insights into the molecular underpinnings of vitiligo. The identification of upregulated and downregulated genes provides a foundation for understanding the dysregulation occurring in vitiligo-affected individuals. Moreover, the exploration of drug targets and potential therapeutic interventions offers hope for future treatments that may mitigate the effects of vitiligo and enhance the management of this complex disease. Continued research efforts in this field are vital to unraveling the underlying mechanisms and developing targeted therapies to improve the lives of those affected by vitiligo.

Chapter 6
Conclusion

In first phase, analysing gene expression of vitiligo. In which we choose samples from GEO and analyse them. Analysation done through graphically representations. Through graphs, up-regulated and down-regulated genes were compared. According to dysregulated of genes, finding out drugs/small molecule which help to stable those genes in their accurate position. The goal of this research was to understand how certain molecules, called ligands, interact with proteins. To achieve this, the researchers used a tool called Protein-Ligand Interaction Profiler. By analysing biological data, they aimed to determine the specific amino acid involved in the attachment of the ligand to the target protein. Through their investigations, the researchers calculated an estimated G value of -4.0, -2.4, -6.6, -3.6, and -4.0 kcal/mol, which represents the energy associated with the binding between the protein and ligand. The docking research provided valuable insights into the relationship between the protein and ligand. Based on the high binding values observed, the researchers concluded that riboflavin is the most suitable choice among the tested ligands. They found that riboflavin exhibited strong binding to the protein, indicating its potential effectiveness for the intended uses. On the other hand, while myristic acid showed average binding values, it demonstrated the ability to cross the blood-brain barrier.

Considering the binding energy, the researchers determined that riboflavin is the best option among the five ligands analysed. This finding is significant because there is currently no definitive medication available for this disorder. The researchers view this as a positive outcome and plan to further explore riboflavin's potential through future experiments.

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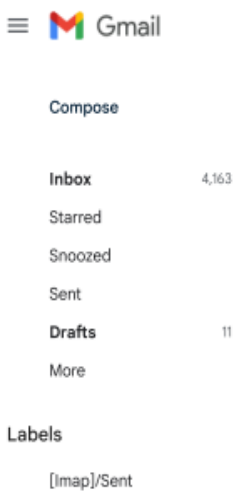

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Conference Paper

In-Silico medication of vitiligo by targeting 6AAH protein and riboflavin Ligand

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***Abstract*— Depigmentation of the skin is a primary symptom of the vitiligo disorder. By reducing their self-esteem and causing them psychological distress, it lowers patients' quality of life. The study made use of a number of computational tools, including Cyto Hubba, BioVia Discovery Studio through, Open babel, Drug bank, Avogadro, Auto dock, and Protein- Interaction Ligand profiler. The interaction between 6AAH and (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) has been examined in this study using Cyto Hubba and PILP clustering interactions, followed by Molecular Docking of Protein and Ligand. Due to its polygenic nature, vitiligo is frequently associated with a number of autoimmune or autoinflammatory disorders, including thyroid disease, psoriasis, atopic dermatitis, diabetes mellitus, and pernicious anaemia. Hence, it is conceivable to think about riboflavin as a possible drug for Vitiligo treatment. The findings imply that riboflavin laboratory tests reveal its inhibitory potential on skin depigmentation**

Keywords*— *gene expression, vitiligo, reversal gene, treatment, depigmentation, drugs.

I. Introduction

Depigmentation of the skin is brought on by the primary pigmentary disorder known as vitiligo. By reducing their self-esteem and causing them psychological distress, it decreases patients' quality of life. The prevalence varies widely by location and is between 0.5% and 2%. Due to a persistent loss of melanin, certain areas of the skin become depigmented. Melanocytes generate the pigment known as melanin. Melanocytes may stop producing melanin in vitiligo or they may even die.[1] An acquired autoimmune disorder is vitiligo. Celsus used the term "vitiligo" for the first time in his well-known Latin work De Medicina in the second century BCE. The Atharvaveda, an ancient work of Indian literature, also makes reference to this illness and describes the horrifying consequences of son-daughter marriage on those who have vitiligo.[2] Hindu writings also claim that stealing garments in a previous life is a potential cause of vitiligo.

Skin consists of three layers: the epidermis, dermis, and subcutaneous tissue. A type of cell found in the dermis is called a melanocyte. [3] These melanocytes create the pigment melanin. Keratinocytes take up melanin in their bodies. Whenever the immune system is activated, melanocytes are decreased or eliminated (autoimmune melanocyte death). Distal extremity tips, segmental, periorificial, and sites of friction are among the body parts where depigmented patches
can be found. [4] Occasionally, asymmetrical depigmented skin may also exist patches that develop on one axil after the other. Macule or patchy skin is described as having no pigment. Compared to a macule, which has a diameter of 10mm, a patch is a flat skin lesion that is larger. The five different kinds of vitiligo are focal, segmental, acro facial, generalized, and universal. Since normal skin and skin with pigmentation can be easily distinguished from one another, these might be easy to spot. Although the exact cause of vitiligo is unknown, there are various theories about how it arises. [5] Here are a few potential explanations: The first is genetics, where genes involved encode a part of the molecular network that controls the immune system and encourage melanocyte death. Second, the immune system of the body targets and destroys melanocytes as a result of an autoimmune response. The immune responses that occur after vitiligo are crucially triggered by oxidative damage. Stressed melanocytes produce DAMPs or autoantigens, which then activate innate immunity and adaptive immunity, leading to melanocyte malfunction and death via an inflammatory cascade Cellular proteins and membrane lipids are impacted by oxidative stress, which is brought on by an increase in reactive oxygen species (ROS) levels and subsequent decrease in antioxidant enzymes, in both lesioned and unlesioned skin.[6] The antioxidant system is therefore less active. Due to its polygenic nature, vitiligo is frequently associated with a number of autoimmune or autoinflammatory disorders, including thyroid disease, psoriasis, atopic dermatitis, diabetes mellitus, and pernicious anaemia. Risk factors include a long family history of vitiligo and genetic vulnerability to depigmentation.

In addition to other techniques, skin biopsies and blood tests can be used to diagnose vitiligo. The production of pigment can be induced by combining herbs Psoralean in pure form with UVA light, often known as PUVA treatment. A tiny area is treated with topical medications. Ultra- or potent topical steroids are used for lesions, whilst mid-potency steroids are used for children.[7] Systemic medications, which also include oral corticosteroid mini-pulses, converted steroid sparing agents, and antioxidants, are a different type of therapy. Another option for

phototherapy is using a blacklight, also known as a Wood's lamp, which exposes skin to UV radiation. Similar to this, localised illnesses are treated using excimer laser therapy, which also uses another laser and additional lighting equipment. Surgical treatment is another option for grafting skin and tissue. [8].

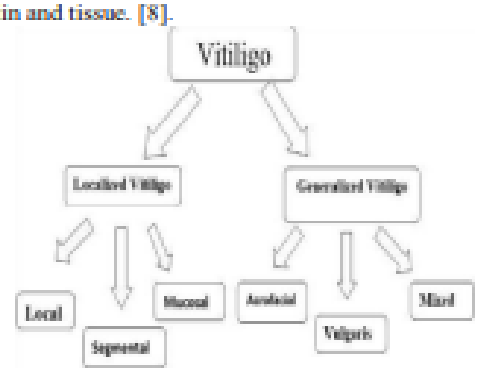

Figure 1.Types of Vitiligo

II. Material and Method

A. Integration of Protein 6AAH-Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl- 7-octene-2,3,6-triol)

The FASTA format of protein 6AAH and Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol were extracted from RCSB PDB (Protein Data Bank) RCSB PDB: Homepage. The PLIP (Protein – Ligand interaction Profiler) web-tool was then used to shows the PLI aggregation. All the ligands successfully bind with the protein showing residual values as 879A, 881B, 889B, 101B,959B respectively. Also find out the amino acids by which they binds are ARG, LEU, VAL, LEU, LEU respectively as above sequence.[9].

B. Docking procedure for proteins or ligands

The heteroatoms, polar hydrogen, and water molecules were carefully taken out. The KOLLMANN charges were received by the ligand and both receptors. The PDBQT file for the agonist or the PDB folder for the receptor were both stored for auto docking. A PDBQT file for agonist is required by auto dock, and this file was converted online using open babel.[10].

C. Used the Auto dock to bind proteins and ligands.

For pharmaceuticals and proteins, there is molecular docking software service. The web server was given the PDB files for the target and the medication, and docking was done by making a grid mapping. Launch the auto-grid file first, then the auto-docking.

D. Uses software called Bio via to do a structural evaluation of the docked protein-drug complex

The result downloads from the auto dock itself were analysed for structural interaction between protein and drug from BioVia Discovery Studio. Submit the complex of protein and ligand to BioVia Discovery Studio and see the interaction with the help of a 2D image. It also helps to analyse by which amino acid ligands bind to the receptor.

E. SWISS ADME examination of the Pharmacodynamic for the ligand.

Absorption, Dispersion, Metabolic activity, and Excretion are combined known as ADME. These variables are examined by adding canonical smiles to study the water solubility, Pharma kinetics, Physiochemical property, lipophilicity, and medical chemistry. These analyses provide evidence for the potency and efficacy of the drug. SWISS ADME (SwissADME). This online tool evaluates the agonist (drug) molecule using these parameters. For the evaluation, the canonical smiles add to sever and run. We can also find out whether the drug crosses the blood-brain barrier or not by seeing egg boiled figure whether the resides inside the yolk (BBB+) or in egg white (BBB-).[11]

III. RESULT AND DISCUSSION

A. Interaction between 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol)

Significant relations between protein 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7-octene-2,3,6-triol) have been observed. All the ligands successfully bind with the protein showing residual values as 879A, 881B, 889B, 101B,959B respectively. Also find out the amino acids by which they binds are ARG, LEU, VAL, LEU, LEU respectively as above sequence. Having hydrophic interaction distances as 2.93 3.83, 4.00, 3.65, 2.37.[12].

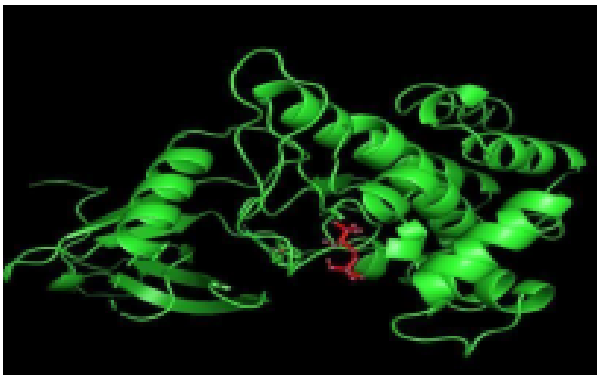

Figure 2. Interaction Of 6AAH and 2,6-Dimethyl-7-octene-2,3,6-triol

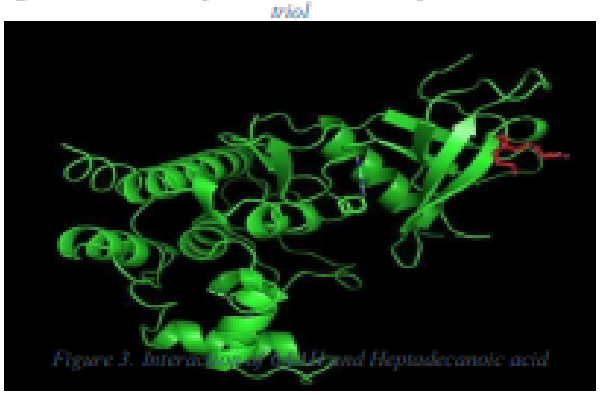

Figure 3. Interaction of 6AAH and Heptadecanoic acid

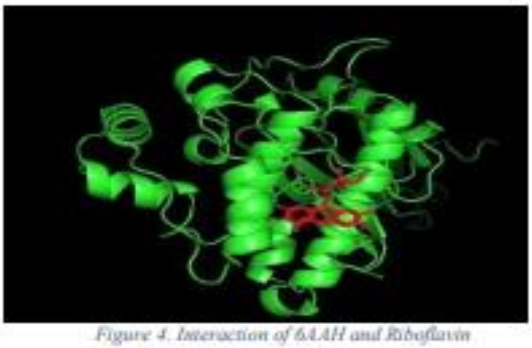

Figure 4. Interaction of 6AAH and Riboflavin

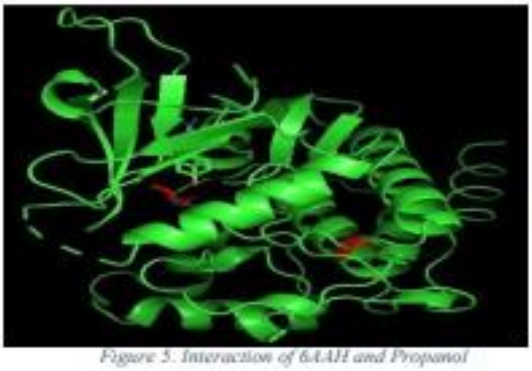

Figure 5. Interaction of 6AAH and Propanol

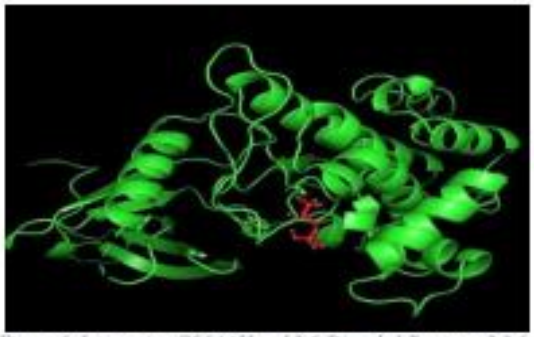

Figure 6. Interaction Of 6AAH and 2,6-Dimethyl-7-octene-2,3,6-triol

B. Interaction between 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl7-octene-2,3,6-triol)

After docking was performed using Auto dock, The outcomes were positive, demonstrating a high level of interaction between 6AAH and Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7- octene-2,3,6triol). The docking scores show that Ligand (Myristic acid, Heptadecanoic acid, Riboflavin, Propanol, 2,6-Dimethyl-7octene-2,3,6-triol) binds efficiently to the 6AAH protein complex. The binding energy is calculated as –4.00kcal/mol, -2.4kcal/mol, - 6.6kcal/mol, -3.6kcal/mol and -4.00kcal/mol respectively and the Cluster RMSD value is 0. This had been analysed via docking results. Docking was used to examine the topology of the molecules' interaction. The structure shows the target protein helical as a green in color consisting of A chain and B chain whereas the potential ligand is represented by the spheroid and the linear structure between the ribbons i.e., ligand. The geometry depicts the interactions of the ligand with the target protein's receptor site. [Fig.2, Fig,3, Fig.4, Fig.5, Fig6]

Table 1. Result of Protein interaction from Swiss

S.No.	Ranks	Ligands
1	Ist	Myristic acid
2	IInd	Heptadecanoic acid
3	IIIrd	2,6-Dimethyl-7-octene-2,3,6-triol
4	IVth	Propanol
5	Vth	Riboflavin

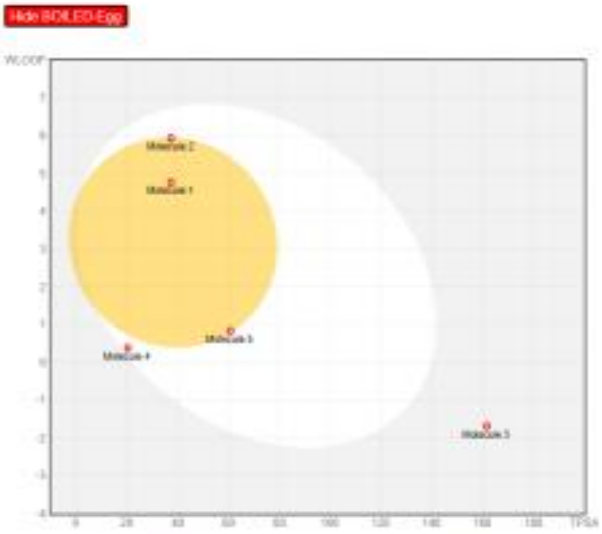

Figure 7. BBB (Blood brain barrier) crossing identified by the help of boiled egg

C. ADME analysis of the ligand

The skin permeation value (log Kp) is low, with values of - 3.35 cm/sec, -2.49 cm/sec, -9.63 cm/sec, -6.49 cm/sec, and - 7.14 cm/sec, respectively. (With Lipinski's value as required and has proven water solubility under logs (ESOL); logs (Ali), and logs (SILICOS-IT) categories with a value of -4.31, -5.37, -1.31, -0.30, -0.95; -6.67, -8.31, -1.43, -0.24, -1.28; - 4.51, 5.71, -2.62, -0.33, -0.72 respectively. The pharma kinetics score of 6.11, 7.69, -1.46, 0.25, and 0.44 and cast log P o/w (XLOGP3) were used to determine the drug's lipophilicity.[13]

IV. CONCLUSION AND FUTURE PROSPECTS:

The aim of this research was to ascertain how ligands and proteins interact. Protein-Ligand Interaction Profiler was utilized in the research. Find out precisely where the ligand

attaches to the target protein by using bio to determine which amino acid is involved. With an estimated G value of -4.0, -2.4, -6.6, -3.6, -4.0kcal/mol, the docking research also demonstrated a notable relationship between protein and ligand value. As a consequence of its high binding values, this finding supports the notion that riboflavin is the best choice for the uses. Although myristic acid has average binding values, it successfully penetrates the blood–brain barrier. Therefore, according to the binding energy, we are able to determine that riboflavin is the greatest option out of all five ligands. We may consider this as a benefit for us and use it further as an experiment in the future since there is no best medication for this disorder.

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CERTIFICATE

I hereby certify that Project Dissertation titled **"Unveiling Dysregulated Gene Expression Analysis in Vitiligo and A Computational Approach for Identifying Promising Drug Targets"** which is submitted by **Sakshi Rajesh Kumar**, Roll No. **2K21/MSCBIO/37**, Department of Biotechnology, Delhi Technological University in partial fulfilment of the requirement for the award of the degree of Master of Technology, is record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere

01.06.23

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Date: 30.5.23

Place: Delhi