In Silico Screening of Natural Compounds against Multiple Disease-Associated Proteins in Vitiligo: A Multi-Target Strategey for Vitiligo therapy

A Dissertation

Submitted in partial fulfilment of the requirement for the degree of

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by:

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The work presented in my thesis, **"In Silico Screening of Natural Compounds against Multiple Disease-Associated Proteins in Vitiligo: A Multi-Target Strategy for Vitiligo therapy,"** which I submitted to the Department of Biotechnology at Delhi Technological University, Delhi, in partial fulfillment of the requirements for the award of the Degree of Master of Science, is an authentic record of my own work conducted under the supervision of Prof. Yasha Hasija from January 2025 to May 2025. I, **Nikhil Chopra**, Roll No. 23/MSCBIO/31, hereby attest that the work is authentic.

I have not submitted the material in the thesis for consideration for any degree from this or any other institution.

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Certified that **Nikhil Chopra (23/MSCBIO/31)** has carried out their search work presented in this thesis entitled **"In Silico Screening of Natural Compounds against Multiple Disease-Associated Proteins in Vitiligo: A Multi-Target Strategey for Vitiligo therapy"** for the award of the degree of Master of Science and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. This thesis embodies results of original work, and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other Institution.

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In Silico Screening of Natural Compounds against Multiple Disease-Associated Proteins in Vitiligo:
A Multi-Target Strategey for Vitiligo therapy

Nikhil Chopra

ABSTRACT

Aim: This study aims to explore the potential of natural phytochemicals in the treatment of vitiligo by targeting multiple disease-associated proteins through in silico methodologies. Vitiligo is a multifactorial autoimmune disorder characterized by the loss of melanocytes, leading to depigmented skin patches.The complexity of its pathogenesis, involving oxidative stress, immune dysregulation, and melanocyte dysfunction, poses significant challenges for effective therapeutic interventions. Emerging evidence suggests that targeting multiple disease-associated proteins simultaneously may offer a more comprehensive therapeutic approach. In this study, we employed an in silico multi-target drug discovery strategy to identify potential natural compounds capable of modulating key proteins implicated in vitiligo pathogenesis. By screening a diverse library of phytochemicals against selected targets such as IFNG, HSP70, and pro-inflammatory cytokines IL-6,IL-23,we aimed to elucidate compounds with favorable binding affinities and pharmacokinetic properties. Subsequent molecular docking, ADMET profiling, and molecular dynamics simulations were conducted to assess the stability and efficacy of these interactions. This comprehensive computational approach aspires to uncover promising natural compounds that can be further developed into effective multi-target therapeutics for vitiligo management.

Results

In this study,selected phytochemicals was screened against vitiligo-associated proteins: Jak1,HSP70,interleukin-6 (IL-6). Molecular docking identified WithaferinA as top candidates out of selected naturalcompounds that exhibit strong binding affinities against each target protein.Subsequent ADMET profiling indicated that these compounds possess favorable pharmacokinetic properties, including high gastrointestinal absorption and compliance with Lipinski's Rule of Five, suggesting good oral bioavailability and safety profiles.This demonstrated to be the best potent inhibitor of Vitiligo associated proteins.

Conclusion

This in silico investigation underscores the potential of natural phytochemicals as multi-target therapeutic agents for vitiligo. Through comprehensive computational analyses—including molecular docking, ADMET profiling —compounds such as WithaferinA demonstrated strong binding affinities(-10.4,-11.5,6.9) Kcal/mol to key targets proteins implicated in vitiligo pathogenesis,Further analysis of the same can be conducted to ratify the computational approach.

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LIST OF ABBREVIATIONS

NSV	Non-Segmental Vitiligo
SV	Segmental Vitiligo
MS	Multiple Sclerosis
RA	Rheumatoid Arthritis
AI	Autoimmune
AIHA	Autoimmune Hemolytic Anemia
Ab	Antibody
HT	Hashimoto's Thyroiditis
GD	GT
AITD	Autoimmune Thyroid Disease

MZ	Monozygotic
DZ	DZ
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4
HSP70	Heat Shock Protein 70
PGAs	Topical prostaglandin analogues
STAT	Signal transducer and activator of transcription
IFN-γ	Interferon-gamma
CXCL9	C-X-C motif chemokine ligand 9
CXCL10	C-X-C motif chemokine ligand 10
ROS	Reactive Oxygen Species
UPR –	Unfolded Protein Response
TRPM2	Transient Receptor Potential Cation Channel Subfamily M Member 2
SOD	Superoxide Dismutase
CSs	Corticosteroids
CIs	CIs
PDB	Protein Data Bank
SIB	Swiss Institute of Bioinformatics
pkCSM	Pharmacokinetics of Small Molecules
CXCR3	C-X-C motif chemokine receptor 3

CHAPTER 1

INTRODUCTION

The condition that causes the skin to lose its color is known as Vitiligo. Depigmented macules and patches of different forms are hallmarks of this autoimmune disease, which lead to the destruction of melanin cointaining cells or damaging their ability to function in the skin. This causes depigment markings on the various parts of the body(hair,skin) . If the region of the skin that is losing color is less than one centimeter broad, the lesion is called a macule; if it is larger, it is called a patch. Vitiligo sufferers, like those with all other skin conditions, are viewed as social outcasts in many civilizations, which has an effect on their mental and physical health.Between 0.06% and 2.28% of people worldwide have vitiligo, and among children and adolescents, the prevalence varies between 0.0 and 2.16%. Vitiligo prevalence varies globally, with higher rates observed in certain regions.. Though few studies have shown a female predominance, both boys and females are equally impacted. Womens are more affected and are more likely to have autoimmune illnesses or because they are more self-conscious about their appearance when seeking advice and treatment. According to the majority of research, half of patients start to exhibit symptoms by the age of 20, and vitiligo usually appears before the age of thirty. A family history may be connected to the condition when it manifests early in children. There are two forms of vitiligo: segmental and non-segmental. Although NSV can manifest at any age, it most commonly affects young individuals between the ages of 10 and 30, with approximately 25% of those who have it developing the condition before turning 10. However, segmental vitiligo can strike 41.3% of patients before the age of ten and manifests earlier than non-segmental vitiligo [1].Vitiligo's precise cause is uncertain. It is commonly linked to a number of autoimmune conditions.Vitiligo is a multifactorial condition characterized by multiple susceptibility loci, genetic variability, and incomplete penetrance. Family and twin studies have demonstrated that its inheritance is complex, involving both genetic and environmental factors. Genetic influences may also affect the age of onset. Genes associated with melanin production, autoantibody regulation, and oxidative stress response are implicated in vitiligo. A variety of complex emotional responses, such as anxiety, sadness, unease, and a decrease in self-confidence, can be brought on by white spots on the skin. These emotions have the capacity to significantly impair a person's overall quality of life, impacting not just the individual but also those in their vicinity.Among the severe psychosocial symptoms of vitiligo that can significantly interfere with day-to-day activities are feelings of embarrassment and paranoia.As a result, sexual relationships may decline, and the consequences may even spread to personal relationship issues and sleep disorders [3].Advancements in computational biology have facilitated the exploration of natural compounds through in silico methods, enabling the prediction of their interactions with multiple disease-associated proteins. Such approaches not only expedite the drug discovery process but also reduce the reliance on extensive laboratory experiments.Multi-target in silico screening approach to identify natural compounds with potential therapeutic effects against vitiligo.

Chapter-2

Review of Literature

2.1 Autoimmune Diseases

AI diseases are a broad category of disorders characterized by an abnormal immune response against the body's own tissues.Specialized cells and proteins (like antibodies) in a healthy immune system defend the body from dangerous invaders like viruses and bacteria. This protective mechanism, however, breaks out in AI illnesses, causing the immune system to wrongly target healthy cells and tissues as though they were alien invaders. Almost every aspect of the body can be impacted by AI disorders, including the neurological system (like MS), joints (like RA), skin (like psoriasis), and organs (such the pancreas in type 1 diabetes). From moderate to incapacitating and life-threatening illnesses, they can differ greatly in their severity and effect on health.People of various ages and ethnicities are impacted by AI illnesses, which together account for a sizeable percentage of the world's population.When the immune system was thought to be targeting the own body tissues in conditions like RA and systemic lupus erythematosus (SLE), the idea of AI disorders started to take shape in the late 19th and early 20th centuries[4].Chronic in nature, AI disorders necessitate lifelong treatment.Better treatments for AI illnesses and perhaps even their prevention depend on an understanding of the mechanisms that cause the immune response to become dysregulated.[5]

2.2 BACKGROUND AND OBJECTIVE

Early in the 20th century, it was suggested that the immune system might identify "foreign"Ag s. The immune system is a tightly controlled biological system that recognizes and reacts to a range of foreign chemicals or organisms, as well as any hazards they may provide, using lymphocytes (white blood cells) and antibodies[4]. Autoimmunity was found by William Dameshek and Steven Schwartz in 1938 while researching AIHA . The idea that an immune response could be triggered not only against foreign Ag s but also "self" Ag s was not recognized until the 1950s.By immunizing guinea pigs with testicular tissue and adjuvants (the Latin word "adjuvare," which means "to support or aid") that postponed the Ag ic stimulus for somatic mutations to occur in the responding lymphocytes, Freund et al. were able to cause AI destruction of the testes. In a similar vein, Witebsky et al. used adjuvants to generate autoimmunity in rabbits by causing AI thyroiditis. Multiple factors, including genetic predispositions, environmental exposures, hormonal impacts, and immune regulatory dysfunctions, interact intricately to cause AI disorders.[5]

Genetics

A mix of environmental and genetic variables contribute to the development of AI illness **(Figure 1)**. It is believed that the majority of AI illnesses are polygenic, meaning they include many genes. Clinical observations that patients frequently indicate a family history of AI disorders gave rise to the theory that people are genetically prone to develop AI disease. For instance, individuals who suffer from HT, GT, or other AITD have a family history of either condition.Several investigations comparing the prevalence of AI disorders in genetically identical MZ twins reported a concordance rate between 10 and 50 percent, while DZ twins showed a concordance rate between 2 and 40 percent. Environmental play an imporatant role in the development of AI illnesses, as evidenced by the poor disease concordance in MZ twins (550%). Therefore, only around one-third of the risk of having an AI disease is due to genetics; the remaining 70% is due to noninherited, environmental factors **(Figure 1).**

Environment

An individual's susceptibility to developing AI illness is influenced by external environmental factors, including hormones, nutrition, medications, chemicals, and/or infections. The chance of developing AI illness can be increased by environmental factors that can breach tolerance in genetically resistant people and increase autoimmunity in genetically vulnerable people **(Figure 2).**

Hormones

In addition to the body's natural synthesis of steroids, hormones can also be acquired from outside sources such as food (such as soy), medications (such as birth control pills), or cosmetics. Natural and synthetic sex hormones directly interact with immune system cells through surface or inside receptors. It is well known that steroid hormones, such as androgens and estrogens, affect the development of antibodies and the growth of immune cells.Hormones can therefore either enhance or suppress the immunological response. Compared to males, women have higher Ab responses, while men frequently experience more severe inflammation.

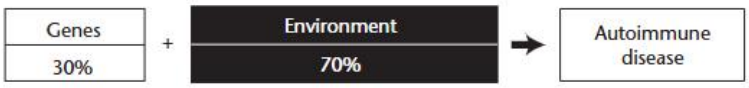

Figure 1 The development of autoimmune disease depends on a combination of genetic and environmental factors like hormones, diet, toxins, drugs and infections. Genetic predisposition accounts for only about one-third of the risk of developing an autoimmune disease, while noninherited environmental factors account for the remaining 70% risk.

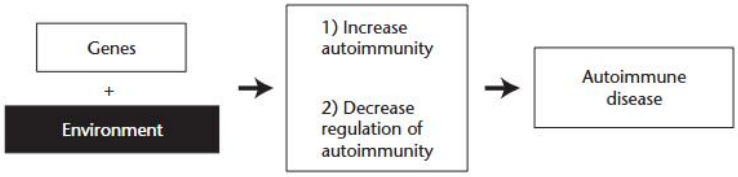

Figure 2 Alterations in mechanisms that regulate inflammation, whether due to genes and/or environment, contribute to the progression from autoimmunity to autoimmune disease. Autoimmune responses are usually generated in the process of mounting an immune response to foreign antigens, but autoimmune disease results only if autoimmunity persists and is poorly regulated.

Regulatory dysfunction of immune response

To preserve immune system homeostasis and avoid or lessen tissue damage, an immune response must be downregulated once it has been induced. Anti-inflammatory cytokines like Interleukin-10 and TGF-b, specialized cells like regulatory T cells, and the inhibitory receptors CTLA-4 and Tim-3 are only a few of the inhibitory mechanisms that control the immune response.It has recently been shown that innate immunity initiates the signals that lead to the immune response's activation and regulation.Therefore, changes in the mechanisms that control inflammation, whether brought on by genes or the environment, may have a role in the development of AI illness from autoimmunity.

2.3 Vitiligo

Vitiligo is a disorder of pigmentation indicated by the development of well-defined, depigmented macules on the skin. Epidermal melanocytes are lost in lesional skin biopsies. Large, depigmented patches may form from lesions that are distributed locally or widely. The condition has a significant influence on the life of both children and adults, and it is particularly deformative in those with darkly pigmented skin due to the contrast between the white patches and normal skin [6]. Vitiligo patients frequently suffer from low self-esteem, social isolation, and stigmatization.

2.4 Classification of Vitiligo

In 2011, the Vitiligo Global Issues Consensus Conference, organized by the Vitiligo European Task Force, proposed a comprehensive classification system for vitiligo. This system categorizes vitiligo into two primary types:

Nonsegmental vitiligo — Generalized, acrofacial or acral, mucosal, and universal subtypes (fig 3). are categorized in NSV. Generalized and acral or acrofacial vitiligo are most common:

Generalized vitiligo – Generalized vitiligo, the most prevalent form of vitiligo, is characterized by the appearance of depigmented macules or patches that are typically symmetrical and distributed across various areas of the body. Beginning in childhood or early adulthood, generalized vitiligo frequently develops at areas that have been impacted by trauma, pressure, or friction. On the face and trunk, depigmented patches are prevalent.

Acrofacial or acral vitiligo – Depigmented macules that are limited to the face and/or distal extremities are the hallmark of acrofacial or acral vitiligo. Later on, it could spread to other parts of the body, leading to the usual widespread vitiligo [6].

Segmental vitiligo — Segmental vitiligo (SV) is a less common form of vitiligo, accounting for approximately 5% of adult cases and up to 20% in children.It is characterized by unilateral depigmented patches that follow a dermatomal or quasidermatomal distribution, frequently aligning with trigeminal nerve region of the face.A notable feature of SV is leukotrichia—the whitening of hair within the affected area—indicating a loss of melanocyte reservoirs in hair follicles.

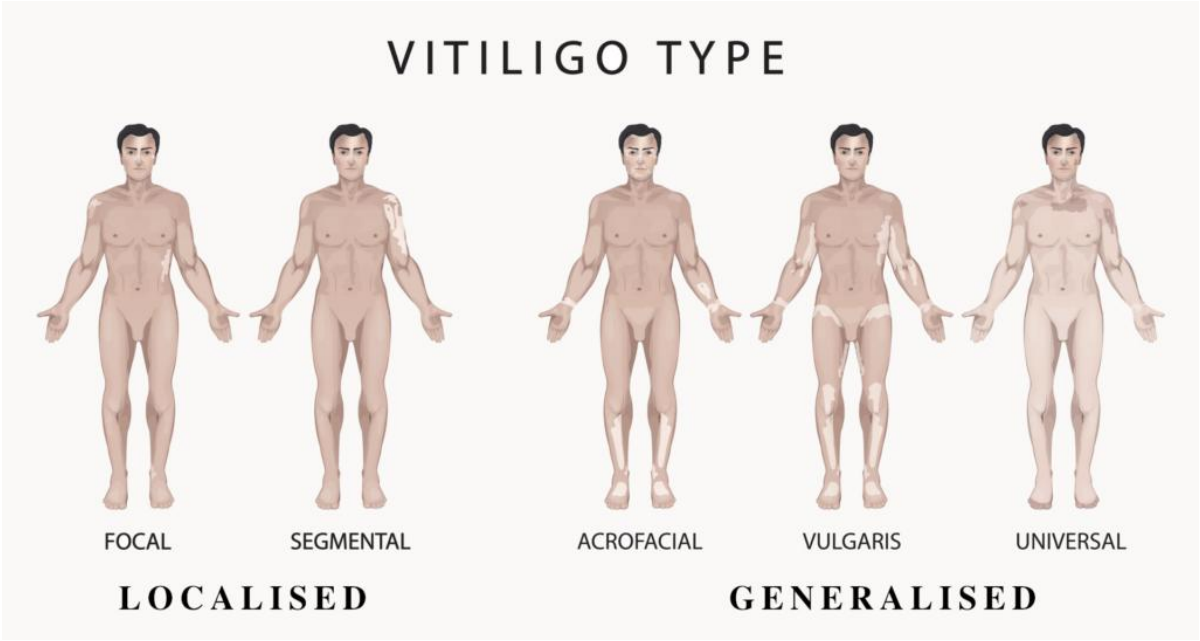

Fig3. Classification of Vitiligo and its pattern

2.5 PATHOGENESIS

There are several hypotheses regarding the demise of melanocytes in vitiligo. These include processes of melanocyte detachment, oxidative stress, viral infection, neurology, AI disease, genetics, and biochemistry. None of the ideas put out can adequately account for the variety of vitiligo phenotypes, despite the fact that the AI and oxidative stress theories are the ones most strongly supported by scientific data.Stress to the melanocytes, the cells that produce the melanin pigment in the skin, can cause vitiligo. Sunburn, mechanical trauma, chemical exposures, and other stimuli all contribute to an immunological reaction that attacks melanocytes and causes progressive skin depigmentation.The following describes each of these causes:

2.6 Autoimmune Theory

It has long been believed that vitiligo etiology heavily involves autoimmunity.Compared to healthy controls, vitiligo patients have higher levels of CD8 + T cells, which are specific to and capable of destroying melanin cointaing cells, and their numbers are correlated with the severity of the condition. The spread of vitiligo lesions was discovered to be significantly influenced by interferon (IFN)-γ 32. In particular, they demonstrated that IFN-γ increased the production of CXCL10, a chemokine that controls CD8 + T cell invasion of follicular and epidermal tissues.Invitiligo, IFN-γ was also found to be a component of a "signature cytokine profile."Since IFN-γ produced by cytotoxic T cells has been shown to induce melanocyte apoptosis, it may have an even more direct function in the pathophysiology of vitiligo.It is becoming more well acknowledged that Interleukin-17 and T helper type 17 (Th17) cells, which produce this cytokine, are crucial for autoimmunity.In a recent thorough assessment of the possible involvement of Th17 in vitiligo, it was discovered that vitiligo is associated with higher levels of IL-17 in the blood, tissue, and cells. In generalized vitiligo, Th17 cell numbers and the cytokines transforming growth factor [TGF]-β and IL-21 were found to be linked with disease activity. As the disease progressed, there was an increase in the production of IL-21 33 and its receptor, IL-21R.The question of whether follicular helper T (Tfh) cells, a recently identified subset of T cells, are involved in vitiligo is raised by the elevated levels of IL-21.Elevated levels of interleukin-21 (IL-21) and its receptor (IL-21R) in vitiligo suggest a potential involvement of follicular helper T (Tfh) cells in the disease's pathogenesis. Tfh cells, distinguished by their expression of CXCR5 and secretion of IL-21, play a pivotal role in B cell activation and antibody production. While humoral immunity is not the primary driver of vitiligo, the presence of melanocyte-specific antibodies indicates a complex interplay between cellular and humoral immune responses. However, these antibodies do not consistently correlate with disease activity, and their uniform distribution fails to explain the characteristic patchy depigmentation observed in vitiligo lesions. This underscores the predominance of cell-mediated immunity, particularly the role of autoreactive T cells, in melanocyte destruction.

2.7 Pathogenesis through the activation of the JAK-STAT pathway.

The chemokine (IFN-γ) axis is crucial to these T cells' function. IFN-γ, which is secreted by T cells, causes keratinocytes to produce the chemokines CXCL9 and CXCL10. The onset, development, and maintenance of vitiligo lesions are caused by these chemokines' increased T-cell recruitment through their binding to the T-cell receptor CXCR3.The JAK-STAT pathway is connected to the IFNγ pathway through its attachment to a specific cell surface receptor (IFNγR), a heterodimeric protein that phosphorylates JAK proteins. JAK proteins phosphorylate STAT, thereby activating it. Phosphorylated STAT proteins move into the nucleus and operate as transcription factors by attaching to DNA, regulating the transcription of several genes, and affecting apoptosis and cell division. This is the physiological basis for JAK inhibitors' ability to effectively treat vitiligo.The durability and recurrence of vitiligo are significantly influenced by resident memory Tcells.Furthermore, numerous studies have demonstrated the significance of resident memory T cells (TRM cells). These CD8+ T cells can stay in tissues and trigger early immunological responses because they express CD69, CD103, and CD49a [8].

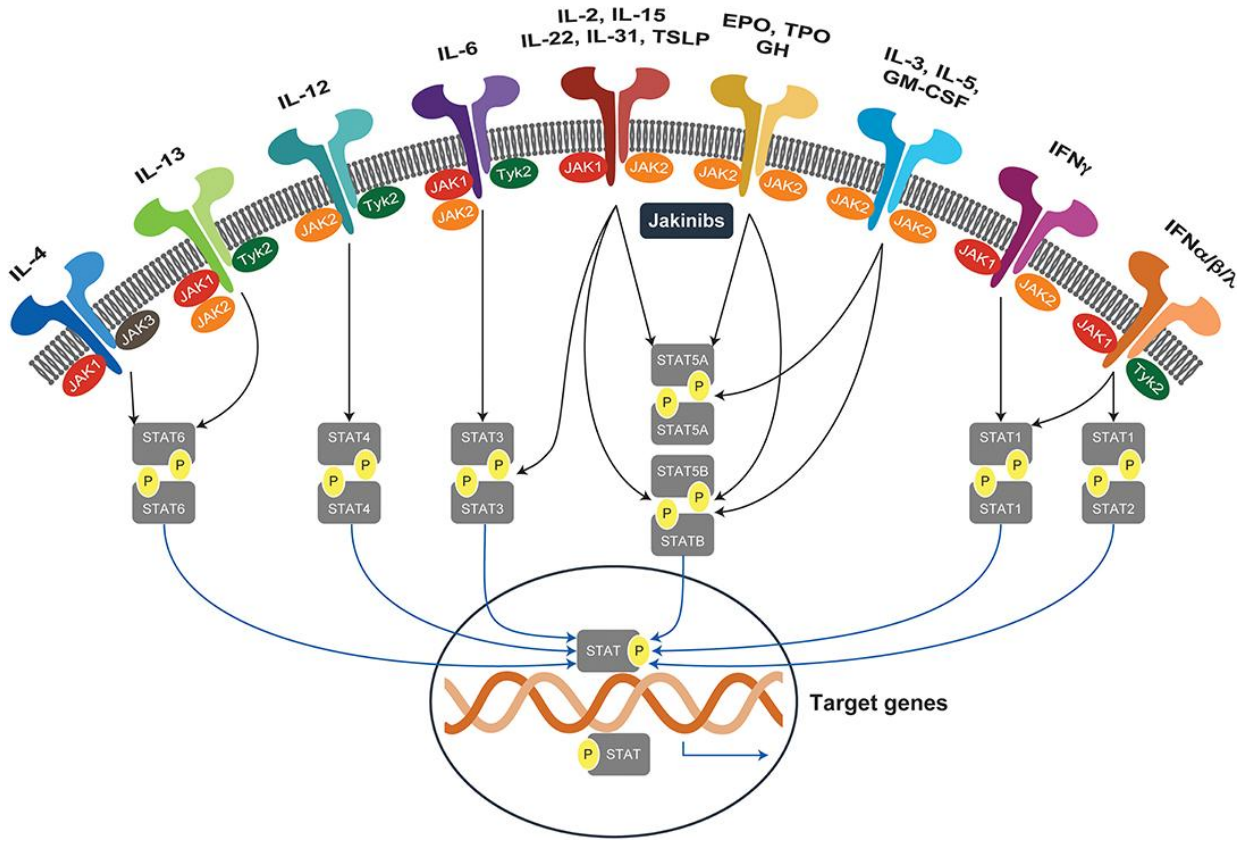

Fig4. JAK-STAT signaling pathways. More than 60 stimuli is responsible for activation of Janus kinases (JAK1-3, TYK2) and phosphorylate downstream STAT proteins, which result in translocate to the nucleus and activate target genes

2.8 Oxidative Stress

Oxidative stress is a key factor in vitiligo's development. Melanocytes from vitiligo patients are more vulnerable to oxidative damage due to inherent antioxidant deficiencies. During melanin production, melanocytes generate reactive oxygen species (ROS), creating a pro-oxidant environment that exacerbates cellular stress. This imbalance between ROS and antioxidant defenses can lead to melanocyte dysfunction and death, initiating depigmentation. Consequently, oxidative stress is considered a primary trigger in vitiligo pathogenesis. An imbalance between pro- and anti-oxidants results from all of this.There is a complete or functional lack of antioxidants, such as catalase, which converts H2O2 into H2O and O2, while pro-oxidant chemicals and enzymes, like superoxide dismutase (which breaks down the O2− radical into H2O2 and O2) and xanthine oxidase, are preferred.The generation of ROS causes defensive molecular pathways to become active.

Melanocytes are subjected to oxidative stress, which modifies the endoplasmic reticulum's protein folding machinery. This results in the buildup of faulty peptides and triggers the "unfolded protein response," a cellular stress phenomena (UPR). Additionally, ROS cause the upregulation of calcium-channel-related proteins that are implicated in mitochondria-dependent melanocyte death, such as

CGRP (calcitonin gene-related peptide) and TRPM2 (transient receptor potential cation channel subfamily M member 2).Melanocyte loss has been shown to be caused by overexpression of iHSP70 in the skin; oxidative stress can also cause an increase in iHSP70 synthesis, that seen in vitiligo melanocytes and may provide a new treatment target.[8]

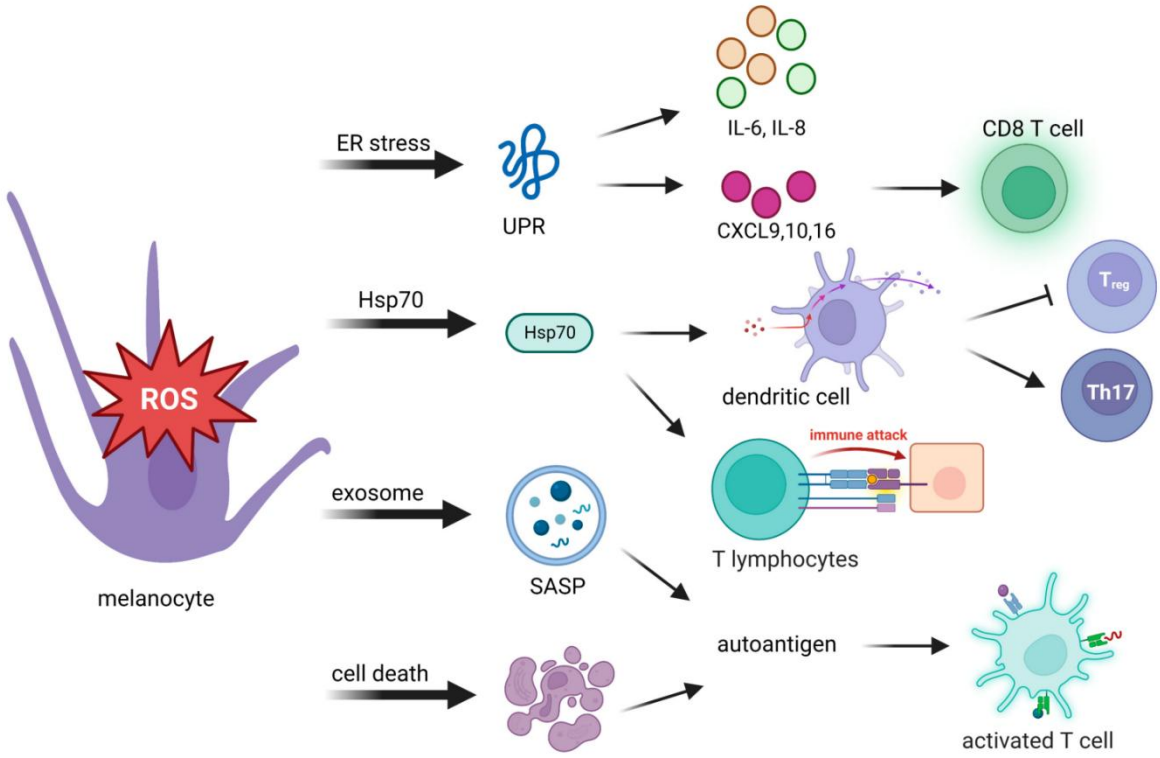

Fig5. Role of Oxidative stress in Vitiligo

2.9 Role of Cytokines in Vitiligo

Cytokines, notably TumorNecrosisFactor-α and Interferon-γ, are elevated in response to cellular damage and play a pivotal role in the progression of vitiligo. These cytokines contribute to melanocyte dysfunction and damage , leading to depigmented skin patches. Understanding the roles of these key cytokines is essential for developing targeted therapies for vitiligo.

INFγ

The primary functions of the cytokine interferon-gamma include immunomodulation, apoptosis, and proliferation inhibition. IFN-γ plays a multifaceted role in the pathogenesis of vitiligo by impacting melanocyte function and survival. It suppresses melanogenesis, elevates reactive oxygen species (ROS) production, and induces apoptosis and senescence in melanocytes, often mediated by CD8+ cytotoxic T lymphocytes. These effects are primarily orchestrated through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway. STAT1 inhibition may also lessen the effects of INFγ on melanocytes, including their senescence. The suppression of Treg by IFNγ may include STAT1 signaling. However, STAT3 is also elevated in vitiligo lesions, indicating that STAT1 is not the only transducer implicated in vitiligo. Interferon-gamma (IFN-γ) plays a pivotal role in the pathogenesis of vitiligo by mediating interactions between keratinocytes and lymphocytes, thereby amplifying inflammatory responses. Upon activation, IFN-γ stimulates

keratinocytes to produce chemokines, notably CXCL10, which serves as a chemoattractant for melanocyte-specific CD8+ cytotoxic T lymphocytes (CTLs). These CTLs express the chemokine receptor CXCR3, and the binding of CXCL10 to CXCR3 facilitates the migration and accumulation of these autoreactive T cells in the skin. This interaction not only contributes to the destruction of melanocytes but also sustains the depigmented state characteristic of vitiligo.Consequently, targeting the IFN-γ/CXCL10/CXCR3 signaling axis indicates promising for the treatment of vitiligo.[9]

TNFα

TNFα activity is increased in active vitiligo lesions. Tumor necrosis factor-alpha (TNF-α) contributes to melanocyte dysfunction and cell death by modulating key regulators of melanogenesis. Specifically, TNF-α downregulates the microphthalmia-associated transcription factor (MITF), reduces melanocyte-stimulating hormone receptor (MSH-R) activity, and diminishes the expression of melanocortin-1 receptor (MC1-R) mRNA. These alterations impair melanin synthesis and compromise melanocyte survival.

IL-6

T cells and macrophages both release IL-6. It has been observed that vitiligo patients, especially those with fresh lesions, have elevated serum levels of IL-6. IL-6 expression may be impacted by oxidative stress. This cytokine is overexpressed by inducing the unfolded protein response (UPR) in the presence of toxic hydrogen peroxide (H2O2) and when melanocytes are exposed to phenols. Therefore, following oxidative damage in vitiligo, Interleukin-6 may contribute to the activation of the immune response.

IL-17

Pro-inflammatory cytokine IL-17 has a direct link to several inflammatory conditions, including psoriasis.Vitiligo patients have greater serum and tissue levels of IL-17 than controls, and there is a positive relationship between Interleukin-17 levels and the surface area of body (BSA) and duration of the condition.By causing monocytic cells to release other pro-inflammatory cytokines proteins such TumorNecrosisFactor-alpha, Interleukin-1β, and Interleukin-6, Interleukin-17 may help the inflammatory network endure [60]. Since IL-17 expression is low in AI vitiligo lesions of SLC, it may be a cytokine that contributes to the development of a process that is initiated by other immunological variables.

2.10 Structure of Target Proteins

Janus Kinase 1 (JAK1)

JAK1 is a tyrosine kinase that transduces signals from various cytokine receptors to the nucleus via the JAK-STAT pathway, influencing gene expression related to cell proliferation, differentiation, and immune responses.

Heat Shock Protein 70 (Hsp70)

Hsp70 is a molecular chaperone that helps in the proper folding of nascent and stress-accumulated misfolded proteins, preventing their aggregation. It plays a crucial role in maintaining cellular proteostasis, especially under stress conditions like heat shock and oxidative stress.

Interleukin-6 (IL-6)

It is a multifunctional cytokine involved in immune regulation, inflammation, hematopoiesis, and metabolism. It plays a pivotal role in the acute phase response and the transition from innate to adaptive immunity.

Fig6. Crystal structure of JAK1 kinase (JH1 domain) in complex with compound 49

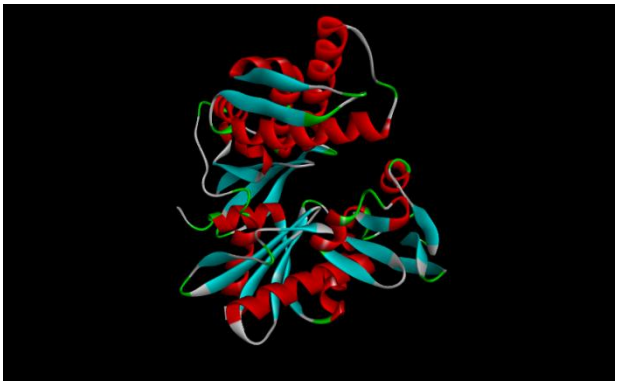

Fig7. Structure of HSp70 kDA in complex with Amitrole

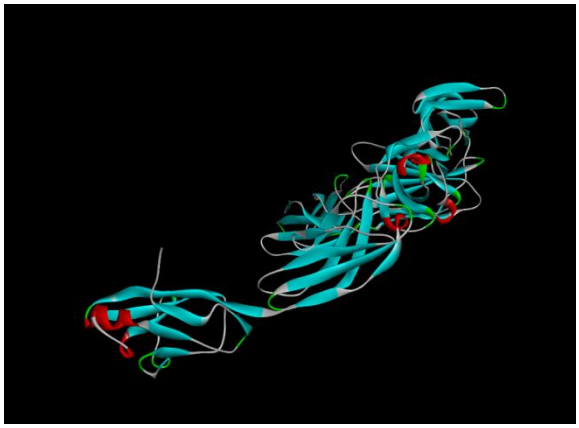

Fig8. Structure of interleukin 6 (gp130 P496L mutant)

2.11 Existing Drugs And Treatment For Vitiligo

According to the European Dermatology Forum consensus guidelines, the initial treatment strategy for segmental vitiligo includes the application of topical corticosteroids or topical calcineurin inhibitors. This approach is complemented by the management of triggering factors and the use of camouflage techniques to enhance cosmetic appearance.

Topical medications

Corticosteroids work by stimulating melanocytes to produce pigment in the afflicted akin and by modulating local immunology. In order to reduce the gene production of several cytokines, including Interleukin 1-6, IL-8, IL-10, IL-13, GMCSF, TumorNecrosisFactor-α, and interferon-γ, they primarily bind to the glucocorticoid receptor pigment (GC receptor). Consequently, cytotoxic T lymphocyte activation is inhibited. Additionally, it has been shown to lessen B cell reactions to self-Ag. Although betamethasone valearate and clobetasol propionate at varying doses have demonstrated good efficacy, prolonged use of these medications is linked to serious local adverse effects. Topical CSs may be somewhat more effective than CIs, and they function better when used in combination than when used alone. [11]

Topical Calcineurin Inhibitors

By blocking Interleukin-2 and IFN-G, topical CIs have an immunomodulation effect on T cytotoxic cells. Tacrolimus has also been demonstrated to lessen systemic oxidative stress, which contributes to vitiligo repigmentation and disease control. In a comparative study against a placebo, tacrolimus demonstrated superior repigmentation outcomes, notably on face and upper back areas, with pigmentation becoming evident within the initial four months of therapy.[11] Because CIs (CIs) have immunomodulatory actions without the side effect profile of CSs, they are beneficial in the treatment of vitiligo. Dendritic cells and lymphocytes contain the intracellular protein calcineurin.It functions as a transcription factor for cytokines including tumor necrosis factor alfa (TNFa) and interleukin-2 (IL-2) when it is activated. 21 IL-10, TNFa, and interferon-gamma are higher in vitiligo sufferers than in healthy controls. Tacrolimus treatment increases the development of melanocytes and melanoblasts while lowering tissue levels of TNFa22. [10].

Prostaglandin analogues

Glaucoma medication causes the periocular skin to become hyperpigmented, PGAs were utilized to treat vitiligo. They are known to induce tyrosinase and enhance the proliferation of melanocytes.Topical PG-E2 gel showed promising results with excellent repigmentation (>75% repigmentation) in 22 patients and complete pigmentation in 6 facial and 2 non-facial lesions.Prostaglandin analogues are better suited for treating periocular vitiligo and other transient disorders. The only side effects were mild itching and burning.[11]

Systemic immunosuppressants

Vitiligo treatment clearly involves immune suppression. The use of systemic immunosuppressants, however, is hardly discussed in the literature outside of CSs.A patient diagnosed with vitiligo

universalis, who had previously shown resistance to systemic steroid treatments, exhibited repigmentation following the administration of cyclophosphamide in combination with dexamethasone pulse therapy for the management of pemphigus vulgaris.

JAK-STAT inhibitors

Tofacitinib

Tofacitinib when taken orally 5mg twice daily has been reported to achieve complete re-pigmentation on vitiligo lesions on the face area and hands in a case of generalised progressive vitiligo after treatment of 5 months.

Ruxolitinib

In a 20-week, open-label pilot study, 11 vitiligo patients with up to 10% body surface area (BSA) involvement applied 1.5% ruxolitinib cream twice daily. Among the nine patients who completed the study, there was a mean improvement of 27% in the Vitiligo Area Scoring Index (VASI), with the most significant repigmentation observed in facial lesions. This suggests that topical ruxolitinib may be particularly effective for treating facial vitiligo.

2.12 Natural compounds for the treatment of Vitiligo

The quantity of secondary metabolites found in medicinal plants, which are biologically active against toxicity and illnesses, has led to their widespread usage in healthcare. Any plant that has compounds that can be used to achieve a therapeutic goal or that provides building blocks for the production of drugs is considered medicinal. Vitiligo has been treated using herbal products of various types and effects [13].

Ginkgo Biloba

Ginkgo biloba, sometimes referred to as the "maidenhair tree," is one of the oldest trees on the planet, and for a very long time, its leaves and seeds have been utilized extensively in medicine. Numerous illnesses, including hypersensitivity , premenstrual syndrome, headaches, vertigo, and others, have been successfully treated using ginkgo extracts. In the past few years, vitiligo has also been treated using gingko extracts. For more than three months, the medication must be taken orally once to three times per day in the form of tablets with varying dosages. Its action appears to be connected to the drug's antioxidant, immunomodulatory, and anti-inflammatory qualities. [14]

Cucumis Melo

The plant species Cucumis melo, commonly referred to as "muskmelon," belongs to the Cucurbitaceae family. The strong superoxide dismutase (SOD) activity shown in cucumis melo extract, which is rich in antioxidants, has been proposed to be crucial in halting the development of melanocytes in the early stages of vitiligo due to oxidative stress. Recent studies have evaluated the efficacy of a topical solution containing Cucumis melo superoxide dismutase (SOD) and catalase in the treatment of vitiligo. [14]

Picrorhiza Kurroa

Using herbal remedies like Picrorhiza kurroa, vitiligo was attempted to be treated in Ayurvedic medicine. Other khellin extracts with well-known hepatoprotective qualities include Picrorhiza kurroa, sometimes referred to as "Kutki" or "Kutaki." More recently, scientists have suggested that the herbal extract also has immune-modulating and antioxidant properties. Potential application of Picrorhiza Kuroda in conjunction with phototherapy for vitiligo treatment. For three months, the medication was taken orally twice daily. Methoxsalen photochemotherapy was administered to patients concurrently. A superior outcome in terms of repigmentation has been shown when the two procedures are combined. [14]

Botanical Name	**Family**	**Part used**	**Chemical Constituents**
Cuscuta chinensis	Convolvulaceae	Stem	Phenolics, steroids and tanins
Adiantum capillus	Polypodiaceae	Leaves and Branches	Glycosides, tannin, resins
Aethae officinalis	Malvaceae	Seeds	Saponins, resins and volatile oils
Berbaris aristata	Berbiaceae	Bark	Berbarin, isobebarin, barbinoids.
Cassia fistula	Caesalpiniaceae	Fruit pulp	Sugar~50 %, anthraquinone
Cassia angustifolia	Caesalpinaceae	Leaves and pods	Glycosides, kaempheral, anthraquinone, chrysophanic acid, isoahamneten, flavones, resin, emodin
Cassia tora	Caesalpiniceae	Seeds	Oleic acid, linoleic acid, palmitic acid and lignoceric acid.
Cinnamomum zeylanicum	Lauraceae	Bark	Eugenol
Citrus colocynthis	Cucurbitaceae	Fruit	Colocynthin, colocynthttin
Crocus sativus	Iridaceae	Flower, Style and Stigma	Colchicines.
Cucumis melo	Curcubitaceae	Fruit	Cucumis melo superoxide dismutase
Cyclonia oblonga	Rosaceae	Seed mucilage	Steroids, glycosides, tannin and volatile oil
Cymbopogon jawarancus	Poaceae	Roots	Citral, citronella glycosides, phenolics and tannins

Foeniculum vulgare	Apiaceae	Fruits and roots.	Fruits contain: anethol, iodine, thiamine, riboflavin, niacin, ascorbic acid.
Glycyrrhiza glabra	Fabaceae	Roots	Glycyrrhizin, glycyrrhizic acid, glycyrrhetic acid, liquiritin, isoliquiritin.
Camellia sinensis	Theaceae	Leaves	Epicatechin, epicatechin-3-gallate, epigallocatechin
Mentha piperata	Lamiaceae	Whole plant	Menthol, esters.
Merremia turpethum	Convolvulaceae	Stems	Glycosidic resin
Nardastachys jatamansi	Valerianceae	Bark	Jatamansic acid
Nigella sativum	Ranunculaceae	Seeds	Alkaloids, glycosides & terpeniods.
Plumbago zeylanica	Plumbaginaceaea	Bark	Alkaloid plumbaginaceae
Prunus dulcis	Poaceae	Kernels	Pentosane, hemicelluloses, oxalic acid, riboflavin, nicotinic acid, folic acid iron.
Psoralea corylifolia	Rutaceace	Fruit	Psoralen, bakuchiole, isobavachin, bavachinin
Pterocarpus santalinus	Caesalpinaceae	Stems	Santalin, pterostillbene, Santalin, pterostillbene, pterocarpin and homopterocarpin

Table 1. Known Natural compounds used for the tratment of Vitiligo[12]

2.13 MOLECULAR DOCKING

A computational approach that uses advanced algorithms to predict how medications will interact with target proteins, which has significant implications for drug discovery and development. This method could reduce the time and expenses involved in drug development by optimizing currently available medications and speeding up the screening of new compounds (Jorgensen, 2004). Since the completion of the human genome project in the early 1980s and the subsequent development of various high-throughput protein purification, crystallography, and nuclear magnetic resonance spectroscopy techniques, which have facilitated and pushed research and development in the development of new therapeutic targets for drug discovery, this computational method has emerged as the most popular approach in structure-based drug design (Kuntz et al., 1982).While early understanding was predicated on Fisher's lock-and-key theory (Fischer, 1894), the ligand-receptor

binding mechanism operates on the same basis as Koshland's induced-fit theory (KOSHLAND, 1963). Our understanding of molecular docking has improved significantly as a result of both hypotheses. Researchers can learn more about the mechanisms of small compounds with potential drug properties within the binding site of particular proteins and the clarification of crucial biochemical mechanisms necessary for improving drug design and efficacy by looking at the interactions between the ligand and receptor at the molecular level (Mcconkey et al., 2002).The ultimate goal is to use a computational approach to investigate the structure of the ligand-receptor complex interaction. This can be accomplished in two interconnected steps: first, ligand conformations from the protein's active site are chosen, and each conformation is then given a score (Meng et al., 2011).

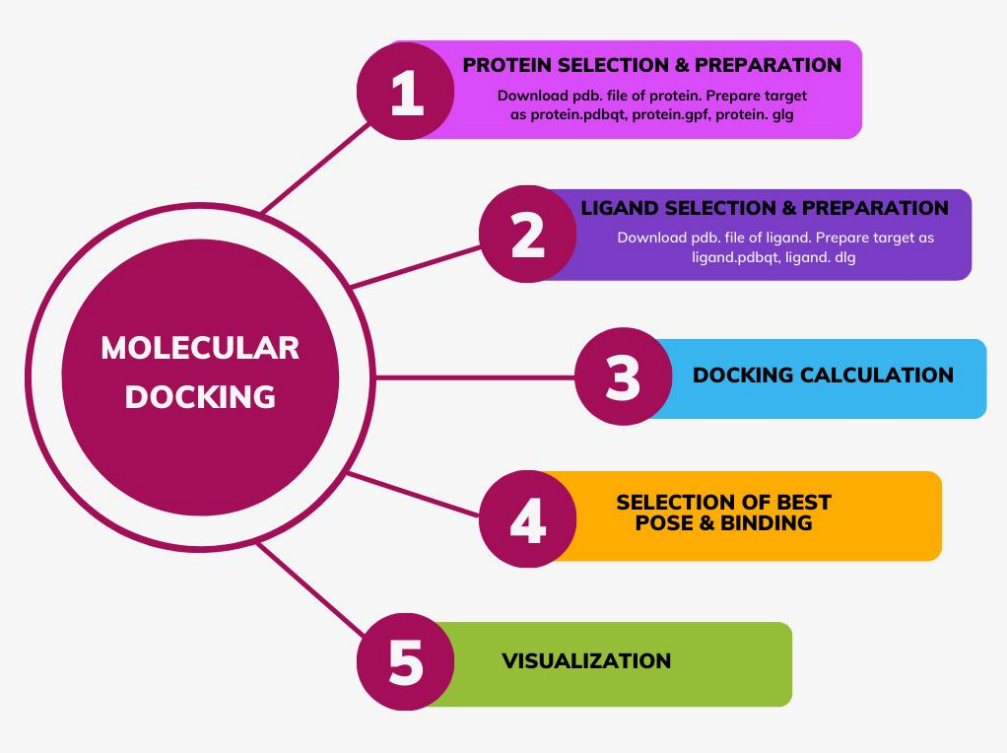

Fig 9. Molecular Docking workflow, which indicates key types

2.14 ADMET ANALYSIS

The pharmacokinetics of a medicine are evaluated by research on ADMET properties . A key component of drug discovery is predicting a drug's fate and the effects it will have on the body, particularly how well it will be absorbed when taken orally and in the gastrointestinal system. Potential drugs frequently fail due to a variety of issues, including insufficient efficacy and safety worries. This emphasizes how vitally important chemical compounds' absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics are at every stage of the drug discovery process. Consequently, a key element of computational drug design is ADMET research (Guan et al., 2019).

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2.14.1 ADSORPTION

For a compound to reach a tissue, it must first enter the bloodstream. Typically, a drug is introduced through mucous membranes like the digestive tract, specifically through intestinal absorption, before it is absorbed by the intended cells. Various factors, such as low solubility of the compound, duration of transit through the intestines, speed of gastric emptying, inability to penetrate the intestinal barrier, and chemical instability in the stomach, contribute to diminishing the level of drug absorption following oral ingestion (Pires et al., 2015).

2.14.2 DISTRIBUTION

Uniform distribution of drugs is essential for the development of highly efficient drugs. The distribution of a drug within the body, from where it is administered to its intended location, is determined by its distribution properties. It is generally thought that smaller molecules with lower molecular weight are more easily transported within the body, making distribution easier. However, this belief has been contradicted by the FDA approval of certain drugs with molecular weights ranging from 500 to 2000 Dalton.

2.14.3 METABOLISM

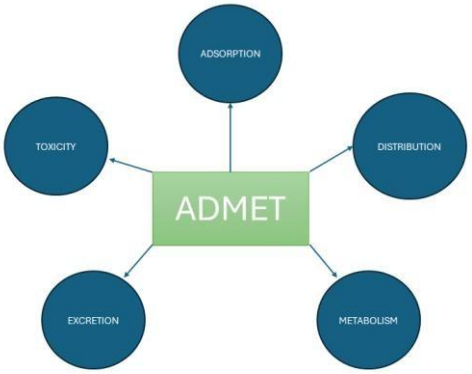


Fig 10 : The various extensively studied ADMET properties essential for drug discovery For a drug to demonstrate its pharmacological effects, it must undergo appropriate metabolic processes which predominantly occurs within the liver.

2.14.4 EXCRETION

Excretion involves eliminating the drug from the body following its activity through the excretory system, commonly through urination. Failure to efficiently excrete a drug postactivity can lead to detrimental effects within the host's organism.

2.14.5 TOXICITY

It is imperative to carefully and precisely address the most important property in question. Toxicity can be defined as any impact of a medication on the recipient's body that deviates from its intended therapeutic effects.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 DATABASES UTILIZED:

Various online databases were utilized to carry out Literature Review, Protein Target acquisition, small molecules (ligands) Library acquisition, and for data retrieval and evaluation

3.1.1 Pubmed (https://pubmed.ncbi.nlm.nih.gov/):

PubMed is an accessible online repository housing more than 33 million references to biomedical literature. Operated by the National Library of Medicine in the United States, it is an indispensable tool for scholars and medical professionals. The database contains a vast array of information on various subjects within biomedicine, health sciences, behavioral sciences, chemistry, and bioengineering.

3.1.2 PDB (https://www.rcsb.org/):

RCSB PDB (RCSB.org) functions as the United States data centre for the worldwide (PDB) to facilitate research and educational activities in fundamental biology, health, energy, and biotechnology by providing access to a comprehensive library of 3D structural data for primary biological molecules (such as proteins, DNA, and RNA).

3.1.3 SwissADME (http://www.swissadme.ch/):

This website enables the calculation of physicochemical descriptors and the prediction of ADME parameters, pharmacokinetic properties, druglikeness, and medicinal chemistry suitability for one or multiple small molecules, in order to aid in drug discovery. Crafted and upheld by the Molecular Modeling Group at the SIB (Daina et al., 2017).

3.14 Gene Cards (https://www.genecards.org/):

All known and predicted human genes are covered in depth by the extensive and user-friendly GeneCards database. GeneCards, created and maintained by the Crown Human Genome Center at the Weizmann Institute of Science in Israel, provides a centralized platform for gene-related data by combining information from more than 150 sources, such as HGNC, Ensembl, and NCBI.

3.2 SOFTWARE UTILIZED

3.2.1 BIOVIA DISCOVERY STUDIO:

A software program called Discovery Studio is used to analyze data, including sequences and molecular structures. Data viewing, editing, and analysis tools are included. A free viewer for opening data from other Discovery Studio products is called Visualizer. It provides an interactive setting for viewing and manipulating many kinds of data. It also gives users the ability to view graphical data representations. Systems, 2016).

3.2.2 PyRx:

PyRx (Python Prescription) is a free and open-source virtual screening software designed for computational drug discovery. It provides an intuitive graphical user interface (GUI) that integrates popular docking engines like AutoDock 4 and AutoDock Vina, enabling researchers to efficiently screen libraries of compounds against potential drug targets.

3.2.3 Auto Dock Vina :

Dr. Oleg Trott from The Scripps Research Institute created the open-source molecular docking program AutoDock Vina. It is frequently used to forecast how small molecules—like possible therapeutic candidates—will attach to a receptor with a known three-dimensional structure. To further speed up computation, the software makes use of multithreading to carry out docking simulations on multi-core CPUs.

3.3 Workflow

The protein JAK1, HSP70, IL-6 was targeting against a comprehensive manually curated database of phytochemicals of traditional Indian medicinal plants, IMPPAT 2.0. Docking based screening of compounds with target proteins was performed to predict their binding affinity and detailed interactions. PyRx, a single-click molecular docking program that automates the entire molecular docking-based virtual screening procedure, was used to do the docking.
To ascertain how the ligands bound to the protein's amino acids, interaction analysis was performed using Biovia Discovery Studio Visualizer. Swiss-ADME and pkCSM were then used to evaluate the best-affinity compounds for favorable ADMET properties. The PASS website was used to predict the biological activity of the best candidate. Additionally, the dynamic behavior and stability of the ligand-receptor complex were assessed using molecular dynamics simulations, which helped choose a viable option for experimental validation.

3.4 Protein and Ligand Prepration

3.4.1 Receptor Prepration

Multiple Proteins associated with Vitiligo such as HSP70(**PDB ID:5fpe)**, JAK1(**PDB ID:4e4n)**, 1L-6**(PDB ID:8qy6),** were selected based on literature studies[7],[2] and their roles in melanocyte dysfunction and immune response. PDB ID of key target proteins is identified.These PDB IDs were used to retrieve the 3D structures of these proteins from the RCSB Protein Data Bank. These structures were created using the Discovery Studio tool by eliminating the water molecule and hetroatoms to the protein structure, adding hydrogen bonds and identifying the protein's binding site.

3.4.2 Active Site Prediction

To understand how a protein works and explore potential treatments, it's important to identify its active binding sites There several computational technique and tools available to predict these binding sites. One such method is FT site (http://ftsite.bu.edu) specifically designed to identify possible locations where a ligand can bind to protein.

3.4.3 Ligand Preparation

The drug smiles downloaded from PubChem were used for various parameter analyses on swissADME. The 3D SDF file can also be downloaded from PubChem. Open Babel is a free and open-source software package that makes it easier to communicate chemical information in various languages, including popular cheminformatics formats such as SMILES, InChl, MOL, and MOL2. The ligands can be converted from SMILES strings into MOL2 forms.Compound were chosen based on their reportes antioxidant and immunomodulatory potential.The 3D structures of selected phytochemical - Withaferin A, Luteolin, Psoralen[15][16] were downloaded in SDF format from PubChem. Thses ligands were then converted to PDB format and energy is minimized using Open Babel to prepare them for docking .To evaluate the relative binding efficiency of the natural compound, known inhibitor were selected for each target protein based on literature reports and database searches:

IL-6:[LMT-28](PubChem CID:49846977) [17]

JAK1:[tert-butyl[(1R,3R)-3-(imidazo[4,5-d]pyrrolo[2,3-b]pyridin-1(6H)-yl)cyclopentyl]carbamate](PubChem CID:53341503)[18]

HSP70:[Apoptozole](PubChem CID:24894064)[19]

3.5 Protein-Ligand Docking Studies

Molecular Docking was performed using Pyrx software to predict the Binding affinity of selected compound and target proteins.During this step all selected natural ligand is docked to each protein to find Binding affinity of selected compounds. Binding affinities were recorded and analyzed to identify the best performing compound across all targets. The best performing compound then comparitive docked to each protein alongside a known refrence inhibitor specific to target.

3.6 Analyze the Structure of the Protein-Ligand Complex

Various molecular visualization software is used to analyze the protein-ligand complex graphically. This analysis help in determining the orientation of the ligand binding site and the overall binding mode and identifying any possible steric conflict.The protein-ligand complexes were analyzed using Discovey studio visualizer to identify hydrogen bonds ,hydrophobic interactions and binding residues . Binding affinity values were compred across all ligand and the best ligand then analyzed to the respective reference inhibitor to evaluate binding interactions and structural compatibility.

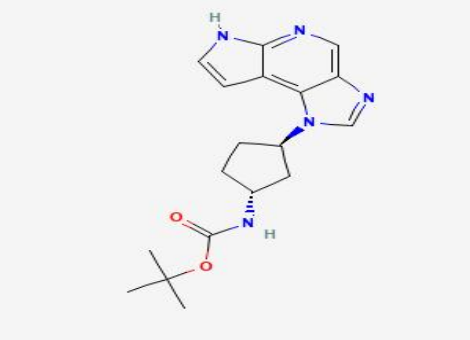
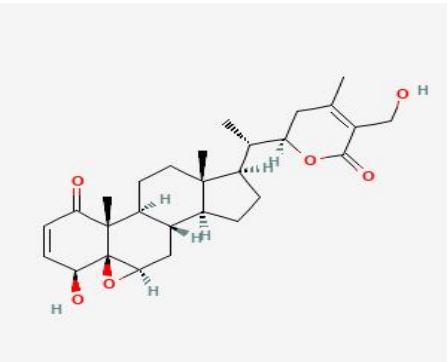
CHAPTER 4

RESULTS

4.1. VIRTUAL SCREENING BASED ON MOLECULAR DOCKING

Screening methods have emerged as a significantly crucial resource for identifying potential drug candidates. Whether employed alongside high-throughput screening or independently, virtual screening offers a rapid and cost-effective approach for identifying new active compounds. Molecular docking-based virtual screening was employed to streamline the selection process, thereby minimizing the in vitro experimentation necessary. Compounds demonstrating noteworthy binding affinity that is greater than the reference drug, Withaferin A were singled out for further investigation after screening against target protein using PyRx .Selected natural compound based on ADMET analysis were prepared and docking is performed to evaluate the binding affinities of selected compound with known reference inhibitor against each target protein. Binding energies are summarised in Table2. Notably WithaferinA exhibits a lowest binding energy of -6.9 kcal/mol in comparable to all the selected ligand Luteolin (-5.5 kcal/mol) Table3 , Psoralen(-5.4 Kcal/mol) Table4. when interact with IL-6. This natural compound show lower binding energy with another target proteins like -10.6 kcal/mol against JAK1 protein,-11.5 kcal/mol against HSP70.These docking score indicate Withiferin A best ligand from all the selected ligands and proceed for competitive docking against reference inhibitor.

Table2. Binding energies against JAK1 with refrence ligand

Compound name	PubChem ID	Binding Energy (kcal/ mol)	Number of hydrogen bonds	Structure
[tert-butyl[(1R,3R)-3-(imidazo[4,5-d]pyrrolo[2,3-b]pyridin-1(6H)-yl)cyclopentyl]carbamate]	53341503	-8.9	2	
WithaferinA	265237	-10.4	1	

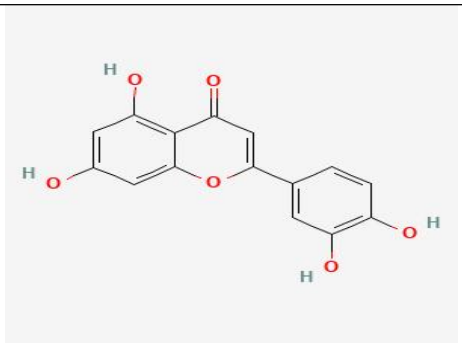
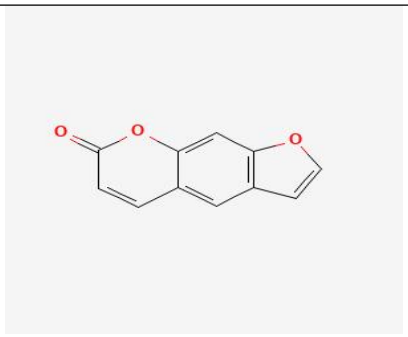
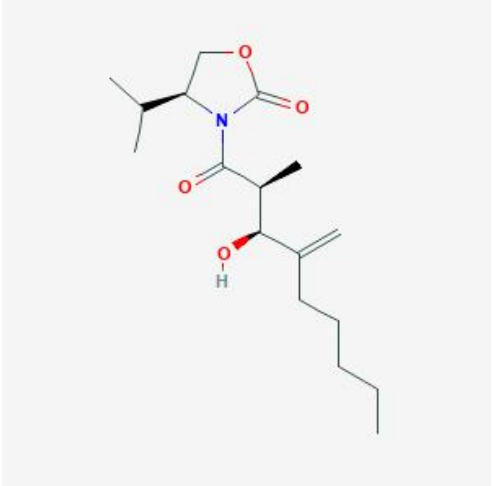
Luteolin	5280445	-9.1	-	
Psoralen	6199	-7.3	-	

Table 3. Binding energies against IL-6 with refrence ligand

Compound name	PubChem ID	Binding Energy (kcal/mol)	Number of hydrogen bonds	Structure
LMT-28	49846977	-4.9	1	

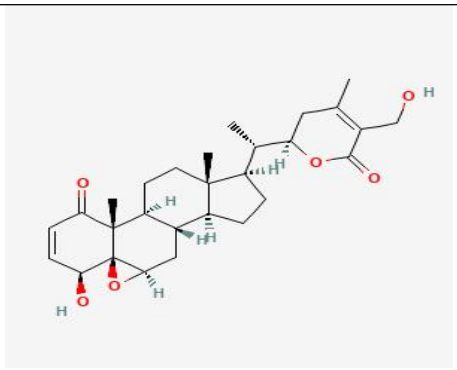
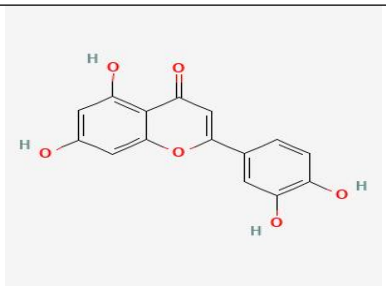
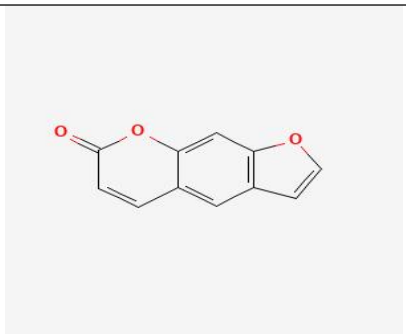
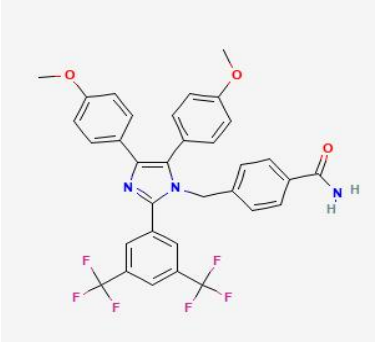
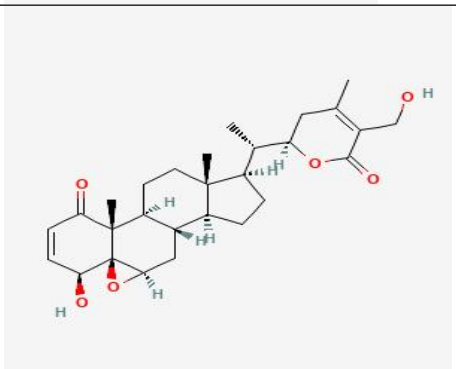
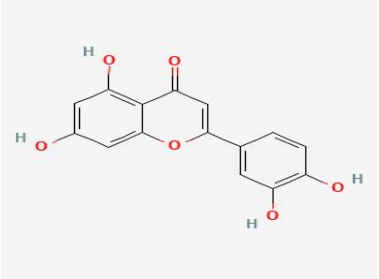
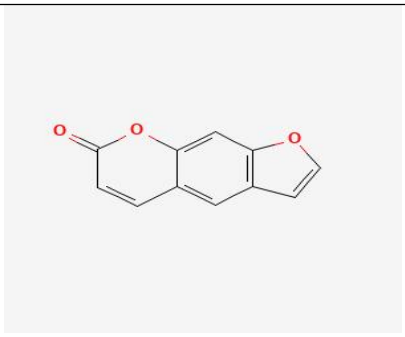
WithaferinA	265237	-6.9	1	
Luteolin	5280445	-5.5	-	
Psoralen	6199	-5.4	-	

Table 4. Binding energies against HSP70 with refrence ligand

Compound name	PubChem ID	Binding Energy (kcal/mol)	Number of hydrogen bonds	Structure

Apoptozole	24894064	-10.2	2	
WithaferinA	265237	-11.5	1	
Luteolin	5280445	-9.0	-	
Psoralen	6199	-6.9	-	

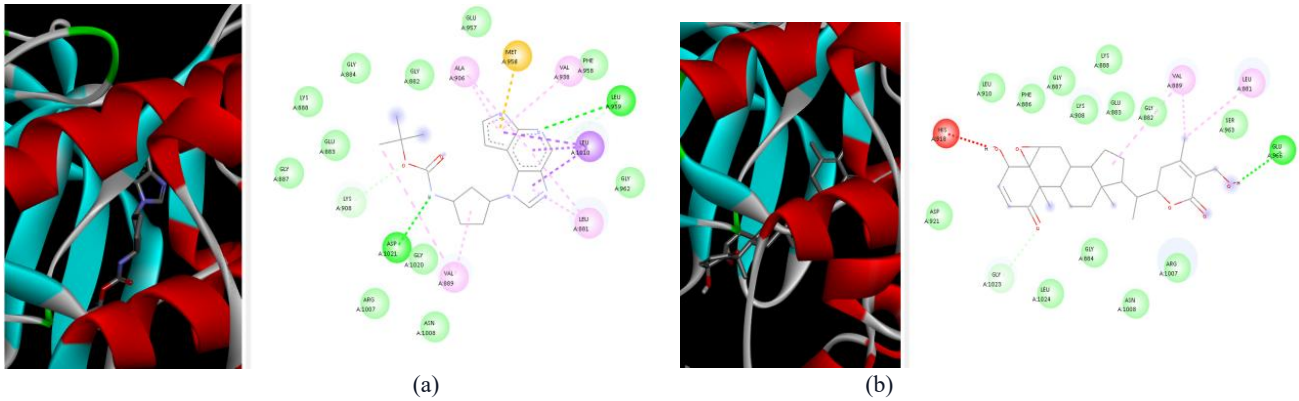
4.2 2D INTERACTION ANALYSIS

2D interaction help us understand and assess the binding of the amino acids on the active site, kinase domain and binding site, all the 8 compounds showed hydrogen bonding in each of the residue. This interaction can help us determine the favourable bonding and binding site. Detailed interaction analysis revealed that WithiferinA formed 1 hydrogen bonds with key residues such as [GLU], similar to the interactions observed with the reference inhibitor.The interaction between the target protein and the ligands is shown in Table 5.

Table 5. 2D interaction analysis ligand molecules with respect to refrence .

Sl. No	PubChem Id	Phytochemicals name	Source of phytochemical	Amino acids involved in binding interactions
1	53341503	[tert-butyl[(1R,3R)-3-(imidazo[4,5-d]pyrrolo[2,3-b]pyridin-1(6H)-yl)cyclopentyl]carbamate]	Synthetic	GLY (962), LEU (1010), ALA(906), MET(958) VAL (938) ,VAL(809)
2	265237	WithaferinA	Withinia Somnifera	GLY (962), LEU (891), ALA(906),

Sl. No	PubChem Id	Phytochemicals name	Source of phytochemical	Amino acids involved in binding interactions
1	49846977	LMT-28 (REF)	oxazolidinone	PRO (327),ARG(326), LYS(450)
2	265237	WithaferinA	Withinia Somnifera	PRO(75),GLN(79) LYS(450)



(a) (b)

Figure 11 : Showing docking results in 2D and 3D structure of (a)[tert-butyl[(1R,3R(imidazo[4,5-d]pyrrolo[2,3b]pyridin1(6H)yl)cyclopentyl]carbamate,(ref) (b) WithaferinA with JAK1

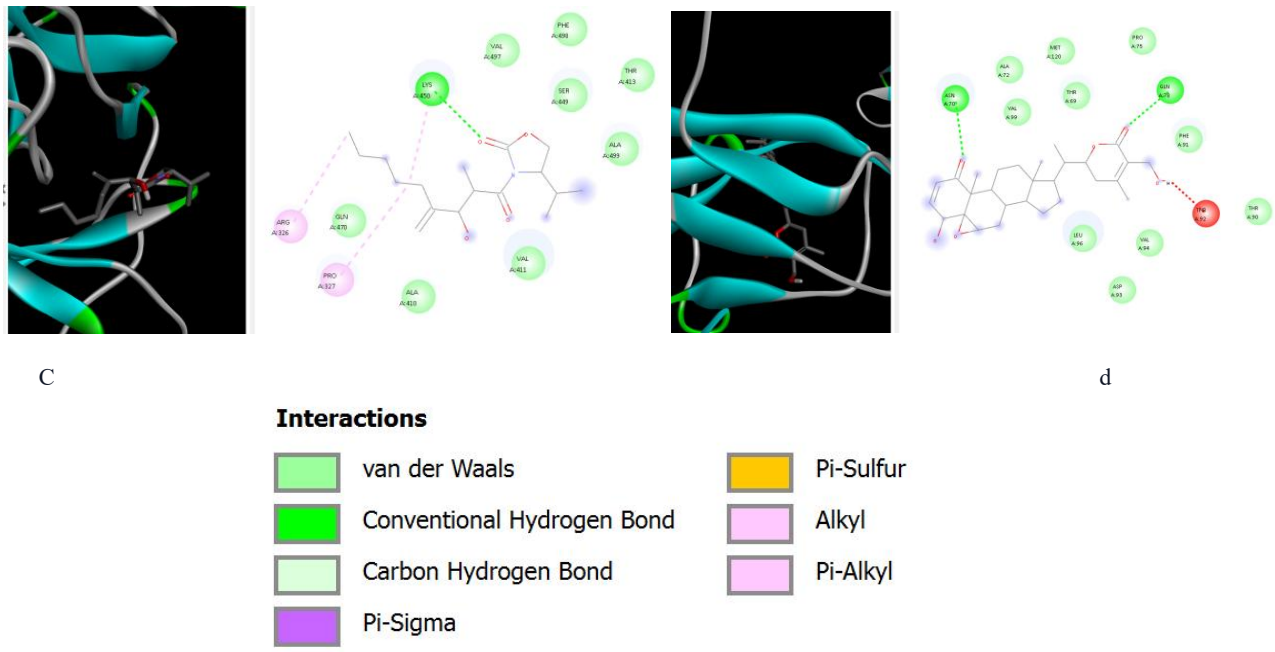
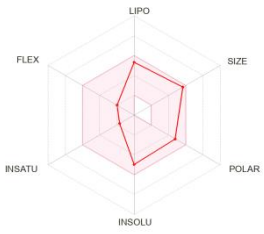


Figure 12 : Showing docking results in 2D and 3D structure of (c) LMT-28 ,(d) WithaferinA with IL-6

4.3 PHARMACOKINETIC ANALYSIS OF LIGANDS

Pharmacokinetic analysis of the obtained ligand was done using Swissadme. The compounds which exhibited all the criteria of potential inhibitor of the targeted protein are listed in the table below, the essential criteria are Lipinski rule of five which has been mentioned in the table II, high GI absorption, TPSA score below 140Å² shows efficacy in oral formulation of these phytochemicals, their bioavailability is 0.55 which makes them suitable substitute in treating Vitiligo. Withaferin A, Luteolin, Psoralen all these ligands shows positive results in ADMET analysis and can be used for further analysis.

Table 6. Lipinski rule of five

Compounds	Molecular weight (g/mol)	Log P	H-bond donors	H bond acceptors	TPSA (Å²)	Lipinski Violation	Bioavailability Radar Illustration
265237	470.60	3.24	2	6	96.36	0	

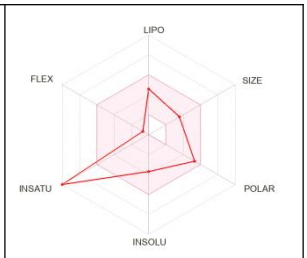
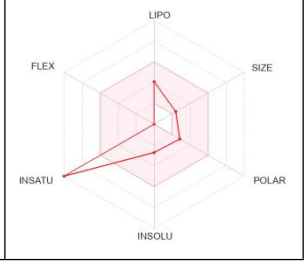
5280445	286.24	1.86	4	6	111.13	0	
6199	186.16	2.01	0	3	43.35	0	

Table 7. In silico Pharmacokinetic analysis of ligands using swiss adme

S.no	Compounds	ADMET Properties				
		BBB Permeabili ty	Solubility	GI Absorption	Drug-likeness Violatio ns	Lead likeness violation
1.	265237	YES	MODERATE	HIGH	NO	YES
2.	5280445	YES	MODERATE	HIGH	NO	YES
3.	6199	YES	MODERATE	HIGH	NO	YES

4.4 BINDING AFFINITY ANALYSIS

The software program (an adaptation of AutoDock Vina) was utilized to determine the binding strengths between the ligand and protein (Hassan et al., 2017). It employs a combination of

empirical and knowledge-based scoring functions in docking computations within an unguided search area for the ligand. The pKi, calculated as the negative decimal logarithm of the inhibition constant, was determined based on the ΔG parameter using the equation:

$$\Delta G = \mathrm{RT}\ (\mathrm{Ln}\ Ki_{\mathrm{pred}})$$

$$Ki\mathrm{pred} = \mathrm{e}(\Delta \mathrm{G}/\mathrm{RT})\ \mathrm{p}Ki = -$$

$$\log\ (Ki_{\mathrm{pred}})$$

Ligand efficiency (LE) serves as a widely used criterion for selecting favourable ligands by comparing the values of average binding energy per atom. The formula employed to determine LE is as follows:

$$\mathrm{LE} = -\Delta G/\mathrm{N}$$

N is the number of non-hydrogen atoms in the ligand, ΔG is the binding affinity in kcal mol-1, and LE is the ligand efficiency in kcal mol-1 non-H atom-1.
In comparison to the other compounds, ebeinone with IMPPAT ID IMPHY000353 has the highest binding affinity with hydrogen bonding in the active site residue, indicating favorable character. In drug development, binding affinity has a major impact on therapeutic potency, selectivity, and efficacy. More potent and efficacious medications with better pharmacokinetic characteristics and possibly fewer adverse effects are typically the result of higher binding affinities.

DISCUSSION AND CONCLUSION

Depigmented skin patches are the result of melanocyte destruction in vitiligo, a complex AI condition. The complicated etiology of the disease may not be addressed by traditional therapeutic techniques, which frequently target single pathways. In order to find more comprehensive treatment candidates, this work used an in silico multi-target approach to find natural compounds that can interact with several proteins implicated in the pathophysiology of vitiligo. In this study potential ligands were identified using ADME analysis. Ligands were docked against refrence inhibitor (ligand) to each protein involved in disease.The molecular docking analysis has show a ligand PubChem ID as 265237 showing lower binding energy than reference ligand and thus exhibiting higher binding affinity towards the respective target protein .This ligands show high Gastrointestinal (GI) permeability, which makes ligands suitable for oral formulation, A TPSA score <140 qualifies them for good absorption. All the ligands have molecular weights under 500g/mol and, TPSA shows a positive correlation with the molecular weight. Compounds with molecular weight higher than 500g/mol show a TPSA score above 140 A[9] The log p-value and bioavailability score higher than zero show efficient cell membrane permeation of the phytochemicals.The log p-value of all the ligands lies between 2-3. The pkCSM analysis of ligands shows a negative Ames test, which shows that all the ligands are nonmutagenic and can show effective results in drug formulation.All the natural ligands follow the criteria of Lipinski's rule of five.

Depigmented skin patches brought on by a decrease in epidermal melanocytes are a hallmark of vitiligo. Autoimmunity plays a part in the development of vitiligo, but oxidative stress may play a part in its start. JAK1, IL-6, and HSP70 protein are important factors in melanocyte depigmentation. It has been observed that HSP70 inactivation reduce autoimmunity activity against melanocytes, JAK1 inhibition pathway works on melanocyte destruction and blocking IL-6 receptor Store repigmentation.They all are major targetsfor formulating a drug, efficiently supporting in treatment of Vitiligo.This study analyzed the binding of three ligands toward the target proteins using molecular docking approaches.s. The ligand WithaferinA having PubChem ID 265237 shows the lowest binding energy and highest binding affinity towards the target protein and form hydeogen bonds Glu966 the intersecting residues. Pharmacokinetic and toxicity analysis of these ligands shows positive results concerning drug likeliness swissADME analysis has shown good absorption and metabolic profiles, the ligand also followed the criteria of Lipinski rule of five and high GI permeation. Toxicity analysis or ames test shows that the ligand is nonmutagenic, which makes it a safer option in drug formulation.

Despite these promising results further analysis needs to be done both in vivo and in vitro. More understanding of the binding between ligands and proteins is needed to analyzed. For a deeper understanding of the binding efficacy between ligand and protein molecular dynamics simulation can also be used. Furthermore, potential off-target inhibitors need to be identified for further use in drug formulation.

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