EXPLORING THE ROLE OF PHYTOCHEMICALS INDUCED JAK2 INHIBITION IN RHEUMATOID ARTHRITIS TREATMENT

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I, Tanisha Shekhawat, Roll no- 2K22/MSCBIO/53 student of Master of science (Biotechnology), hereby declare that the project dissertation titled "Exploring the role of Phytochemicals induced JAK2 inhibition in Rheumatoid Arthritis treatment" which is submitted by me to the Department of Biotechnology – Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, 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 the project dissertation titled "Exploring the role of Phytochemicals induced JAK2 inhibition in Rheumatoid Arthritis treatment" which is submitted by Tanisha Shekhawat, Roll no. 2K22/MSCBIO/53, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of master of science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or fully for any degree or diploma to this university or elsewhere.

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ABSTRACT

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that mostly affects the joints, and causes the weakening of tendons and ligaments. The common symptoms of RA include tender joints, fever, fatigue, and often rheumatoid nodules under the skin. JAK2 is a tyrosine kinase that regulates the expression of pro-inflammatory cytokines. Persistent activation of the JAK-STAT pathway is due to the dysregulation of cytokine signaling contributing to synovial inflammation. Therefore, JAK2 inhibitors are required to treat the underlying disease rather than only curing symptoms. The phytochemicals such as ellagic acid, quercetin, curcumin and andrographolide have shown anti-inflammatory effects through modulation of the JAK-STAT pathway. These phytochemicals inhibits the phosphorylation of JAK2, which in turn reduces STAT transcription protein phosphorylation, regulating the overall JAK/STAT signalling, thus attenuating inflammation. So, for this we use ligands i.e. ellagic acid, quercetin, curcumin etc. which inhibits the activity of JAK2 protein, thus reducing the symptoms of RA. We are essentially assessing the interaction between the ligand and the protein while working on molecular docking. In-silico, assays demonstrated that curcumin reduced arthritis scores and enhanced inflammatory infiltration. Docking studies can show the binding affinity between ligands and proteins, thus providing predictions on the strength of their potential interaction. So, using molecular docking we infer that phytochemicals taken have a higher negative binding affinity than approved JAK inhibitor tofacitinib and can target RA symptoms.

Key words- Rheumatoid Arthritis, Phytochemicals, JAK2, JAK-STAT pathway, Molecular Docking

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LIST OF ABBREVIATIONS

RA	Rheumatoid Arthritis
JAK2	Janus Kinase 2
MAPK	Mitogen-Activated Protein Kinase
ERK1/2	Extracellular Signal-Regulated Kinase
AP-1	Activator Protein-1
NF-κB	Nuclear Factor-Kappa B
TNF-α	Tumour Necrosis Factor-Alpha
IL-6	Interleukin-6
IL-10	Interleukin-10
EGF	Epidermal Growth Factor
JAKs	Janus Kinases
PDB	Protein Data Bank
FLS	Fibroblast-Like Synoviocytes
MMPs	Matrix Metalloproteinases
DMARDs	Disease-Modifying Antirheumatic Medications
DMSO	Dimethyl Sulfoxide
PI3K	Phosphoinositide 3-Kinase
CIA	Collagen-Induced Arthritis
SAR	Structure-Activity Relationships
SMILES	Simplified Molecular-Input Line-Entry System
SDF	Structure Data File

GI	Gastrointestinal

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CHAPTER 1

INTRODUCTION

The systemic chronic inflammatory illness known as rheumatoid arthritis (RA) gradually destroys the synovial lining of joints. Pain, loss of voluntary function, deformity, and a general reduction in quality of life can result from RA if treatment is not received. Methotrexate (MTX) has long been the mainstay anti-rheumatic drug (DMARD) for disease-modification and the treatment of RA. This is because patients do not always experience the desired outcomes from MTX use; consequently, a significant number of patients stop treatment within the first year owing to ineffectiveness or side effects. In order to create and launch more potent treatments and alternatives, there has been a continuous endeavour to comprehend the pathophysiology of RA and investigate alternative therapeutic targets.

Many biologic disease-modifying antiretroviral (bDMARDs) have been developed recently as potential treatments. These offer a more focused type of immunosuppression than methotrexate (MTX), which has a much broader target and lower efficacy. Despite the development of multiple treatment options, for example interleukin 6 (IL-6) inhibitors as well as tumor necrosis factor (TNF) inhibitors, and B cell depletion targeting CD20 cells, a majority of RA patients continue to experience intolerance to current bDMARDs, suboptimal control, and loss of response.

Recently, there has been a significant increased usage of cytokine targeting for the treatment of autoimmune illnesses such as rheumatoid arthritis (RA) and other conditions. The effectiveness of biologics for RA and other associated conditions has been studied using basic animal models, and recent research has fundamentally altered the therapy approaches. Only about 30% of RA patients who have undergone biologic treatment attain complete remission, despite the fact that early intervention can somewhat improve disease outcomes. Even among the patients who had reacted well to treatment, the condition worsened by roughly 50–80%, demonstrating the need for long-term biologic use to stop the disease from getting worse.

The most recent medication to treat RA is called a Janus kinase (JAK) inhibitor. Tofacitinib, baricitinib, upadacitinib and peficitinib are some of the approved JAK inhibitors. There are truly clinically viable oral small-molecule therapies for RA that are also widely available for the first

time. These medications can be given orally and offer long-term advantages. Research on a potential targeted therapy for RA has benefited from findings that demonstrate the significance of JAK and signal transducer and activator of transcription (STAT) in signalling of cytokines and ultimately RA pathogenesis JAK inhibitors are much smaller molecules and may readily pass through the lipid bilayer of the cell membrane. JAK inhibitors were favoured in the first phase due to their specific mode of action and oral delivery simplicity. It was largely preferred by the patients A significant number of the therapy's adverse effect considerations, such as the risk of venous thromboembolism (VTE) and the incidence of herpes zoster infection, are alarming, nevertheless.[1]

These drawbacks emphasize the need to investigate innovative natural origin JAK/STAT inhibitors that provide improved cost-effectiveness, safety, and tolerance. This study will give an overview of several natural compounds and phytochemicals that have been demonstrated to help reduce inflammation in RA by modulating the JAK/STAT signalling pathway. Among these, substances such as curcumin have demonstrated important therapeutic and biochemical benefits in RA amelioration, both in vivo and in clinical settings. In summary, the potential of natural JAK/STAT inhibitors to alleviate RA at a reasonable cost and without the possibility of serious side effects will be covered, opening the door for further research into these compounds as adjunctive therapies to current RA medications.[2]

1.1 Rheumatoid arthritis

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation. In addition to having a negative impact on the kidneys, heart, digestive tract, lungs, skin, eyes, and nervous system, inflammation can also negatively impact the joints. The examination of various forms of arthritis led to their classification as either non-inflammatory (e.g., osteoarthritis) or inflammatory (e.g., gout, pseudogout, basic calcium phosphate disease), or as a result of an autoimmune mechanism or bacterial or viral infections (e.g., Staphylococcus aureus, Neisseria gonorrhoea, enterovirus, Parvovirus, complications of Lyme disease). [3]

About 0.4% to 1.3% of people have RA, and the prevalence varies depending on factors like age (new RA diagnoses are most frequently found in the sixth decade of life), gender (women are affected by RA 2-3 times more frequently than men), and demographics of the population under

study (RA frequency usually from south to north and is most prevalent in the urban areas as compared to rural areas). As a result, RA distinguishes itself as one of the most prevalent chronic inflammatory diseases. The clinical symptoms of RA vary significantly between the disease's early and late stages, which are poorly treated. Typical early-stage RA symptoms include weariness, flu-like symptoms, sore and swollen joints, and stiffness in the mornings. Elevated C-reactive protein (CRP) is frequently present in conjunction with these symptoms along with an increase in the erythrocyte sedimentation rate (ESR). However, inadequately treated RA has a much more complicated clinical picture including the development of major systemic manifestations such as lymphomas, keratoconjunctivitis, hematologic abnormalities (e.g. leukopenia, neutropenia, eosinophilia, or thrombocytosis), joint malalignment, reduced range of motion, cartilage degradation, and rheumatic nodules. It may be concluded that these systemic manifestations are mostly brought on by the chronic inflammatory state that RA patients experience, and they also play a role in the higher death rates. [4]

The single membrane synovium in RA synovial joints experiences hyperplasia. This modification is caused by increased levels of different chemokines and adhesion proteins, which function as guides for the movement and adherence of activated immune and non-immune cells. Additionally, pro-inflammatory cytokines with significantly elevated levels for example tumor necrosis factor-α , and interleukins (IL) -1ß, -6, -7, -8, -12/IL-23, -15, -17, -18, and -32—also growth factors like fibroblast growth factor—2 (FGF-2) along with vascular endothelial growth factor—which are primarily synthesized by synovial-like fibroblasts and macrophages—are produced by various RA-affected cells. These factors play crucial roles in the clinical progression of RA and ultimately result in the primary events of articular cartilage destruction and subchondral bone erosion, which further leads to synovial joint failure.

Given that women make up around 75% of RA patients, hormones have a significant role in the etiology of the disease. Stress and smoking are also thought to be significant causes of RA, which is characterized by symptoms including swelling and stiffness in the joints, frequently on both sides of the body in a systematic manner. Patients with RA can reduce their level of impairment, manage their pain and swelling, slow down the progression of the disease, and generally live better thanks to these techniques. Treatments such as NSAIDs, opioids, and analgesics like acetaminophen, as well as intra-articular medications like glucocorticoids, can be

used to manage pain and swelling. Moreover, DMARDs, or disease-modifying anti-rheumatic medications, are utilized to modulate the radiological and clinical course of RA. Sulfasalazine, Methotrexate (MTX), hydroxychloroquine, and more recent treatments like abatacept, infliximab, and etanercept, as well as anti-tumor necrosis factor (TNF)-α and anti-CD20 medicines such as Etanercept and Rituximab are examples of DMARDs. All of these medications do, however, have a long list of negative effects.[5]

1.2 Role of JAK-STAT signalling pathway in RA

The JAK-STAT system plays a vital role in cytokine signaling, notably in TNF-α's fast activation of target genes. These include the interferon, gp130, and common-γ chain families, receptor tyrosine kinases, and several G protein-coupled receptors. They can initiate transduction via the JAK-STAT pathway.[6] This route is essential for cell differentiation, apoptosis, immunological function and proliferation; it plays a major role in controlling inflammation and immunity. Recent research has revealed that the JAK-STAT signaling system is abnormally active in rheumatoid arthritis.

The JAK family encompasses four members: JAK1, JAK2, JAK3, and TyK2. These members' constituents of different molecular weights but are largely preserved throughout evolution. JAK3 is expressed particularly in vascular smooth muscle, endothelial cells, and blood, on the other hand JAK1, JAK2, and TyK2 are extensively distributed in a different tissues and systems of our body.As per the research done in the past, JAK plays major function in RA.[7]

STAT is a cytoplasmic protein family which regulates the transcription and signal transmission in various signalling pathways.STAT1-4, STAT5B, STAT6 and STAT5A are members of the STAT family, and each contain six functional domains which are highly conserved; the helix domain, the DNA-binding domain, the SH2 domain, the ligation domain, the C-terminal transcription activation domain and the N-terminal conserved domain. The SH2 domain is the most conserved and functionally crucial, It enables the specific detection and docking of phosphorylated tyrosines on cytokine receptors, JAKs, and other molecules of STAT. The N-terminal regulates interactions with other transcription factors, whereas the DNA-binding domain controls DNA interaction.[6]

Recent years have seen a strong correlation between the abnormal activation of the JAK-STAT pathway and the development and progression of RA. This pathway is implicated in various pathological conditions, contributing to the abnormal proliferation of RA fibroblast-like synovial inflammation, bone destruction and synoviocytes (FLS). Synovitis, the pathological foundation of RA, leads to the abnormal proliferation of synovial tissue, resulting in bone and cartilage destruction. Inflammatory responses in RA synoviums, such as adhesion molecule gene and cytokine activation, are significantly linked to signaling pathway transcription factors. [8]

1.3 Phytochemicals for treating rheumatoid arthritis

A wide range of plant-derived natural substances (phytochemicals) with pharmacological and biological properties promising as potential JAK inhibitors have been identified from medicinal plants. Secondary metabolites such as polyphenols, alkaloids, terpenoids and flavonoids have shown extreme usefulness in the development of treatments for many diseases such as cancer, Parkinson's, rheumatoid arthritis, and some cardiovascular disease. [9]As a result, natural chemical derivatives of plants have great medicinal value and promising potential as a treatment for inflammatory disorders such as arthritis.

Phytochemicals have anti-inflammatory and immunomodulatory effects which are contributed through various mechanisms, such as pro-inflammatory enzyme modulation activities like cyclooxygenase (COX), lipoxygenase (LOX),antioxidant and radical scavenging activities, it also inhibit cytokines such as TNF-α, IL-1β, and IL-6, modulate inflammatory mediators, and modulate pro-inflammatory signaling pathways including NF-κB. Phytochemicals are particularly important because of their ability to suppress the activity and expression of pro-inflammatory mediators and transcription factors associated with inflammation and autoimmune disorders.[10]

Such phytochemical substances are usually found in grains, herbs, fruits, vegetables, , berries, legumes and tea, which are safe to eat by humans, suggesting minimal toxicity. Plant-derived natural substances with anti-inflammatory, anti-arthritic and immunosuppressive characteristics include quercetin, curcumin, Andrographolide, ellagic acid.[11]

1.4 Basis for utilizing plant-derived phytochemicals as JAK/STAT inhibitors in RA

The previous sections that were discussed provided enough scientific evidence for the toxic consequences and high risks of serious infections that can be a result of the use of traditional small molecule JAK/STAT inhibitors in therapy for RA. Additionally treatments that are available are expensive, limiting the availability, especially in underdeveloped countries. The annual cost of tofacitinib 5 mg in the United States is very high.

Due to all listed limitations of the therapy options that are present for RA the discovery of new JAK/STAT inhibitors from natural sources, such as flavonoids, alkaloids, catechins, terpenoids, and isoflavones becomes a demand. The use of phytochemicals with JAK/STAT inhibitory activity is particularly beneficial due to many reasons including their simplicity of oral administration, cost-effectiveness, higher acceptance, and favorable safety profile. Natural chemicals have a long history of usage in treating numerous autoimmune conditions, including RA, multiple sclerosis and inflammatory bowel disease. However, they are generally underestimated as therapeutic agents.[12]

Studies have shown that plant-derived natural agents such as curcumin, ellagic acid, quercetin, resveratrol, berberine and andrographolide have anti-inflammatory and anti-arthritic properties. Further, various herbal medicines, have also been used in traditional Chinese medicine (TCM) to treat RA due to their immunomodulatory and anti-inflammatory characteristics. Several of these natural substances have been studied as adjuvants in conjunction with conventional DMARD treatment in RA patients, for example resveratrol combined with conventional DMARDs have shown significant improvement in orevious studies with much lower side effects. Thus it is implied that efficacy and tolerance both increases signifantly by use of natural compounds.

Therefore, targeting the JAK/STAT signaling system using natural substances or phytochemicals appears to be a plausible therapeutic method for treating autoimmune illnesses such as RA. In this study natural compounds having demonstration of promising results in alleviating RA symptoms in the past, particularly through their immunomodulatory effects, especially by targeting the JAK/STAT signaling pathway would be highlighted further. [13]

CHAPTER 2

LITERATURE REVIEW

2.1 An overview of Rheumatoid Arthritis

2.1.1 Pathogenesis of RA

Initial pathogenesis of RA includes a condition of continues cellular activation, resulted due to autoimmune affects in joints or some other organs as well. The clinical signs of the illness are predominantly due to synovial inflammation and joint damage. Fibroblast-like synoviocytes (FLS) play a crucial role in degenerative processes. The course of RA constitutes total of three different stages: non-specific inflammatory stage in the initial phase, which is enhanced by T-cell activation in the synovium; a chronic inflammatory stage; and a tissue damage stage, which is controlled by cytokines including IL-1, TNF-α, and IL-6. [8]

-Inflammation and Autoimmunity

Autoantibody generation in RA is frequently associated with severe symptoms, such as joint destruction and greater mortality, which is produced by immunological complexes, which are caused by autoantibodies against citrullinated peptides (ACPAs) and citrulline-containing antigens. These complexes connect to rheumatoid factors (RF), resulting in complement activation.

Joint swelling in RA is due to immune activation-induced synovial inflammation, which is defined by the influx of leukocytes into the synovial compartment. Various factors could be responsible for this including Environmental or genetic variables which stimulate dendritic cells and activate innate immunity. DCs attract and cause activation of T cells, further exciting B cells, macrophages and osteoclasts, resulting in the release of pro-inflammatory and bone-destroying cytokines, and matrix metalloproteinases (MMPs). Therefore this interaction of innate and adaptive immunological mechanisms in the bone marrow and synovium can be considered as major cause of tissue damage, causing persistent inflammation in RA. This mechanism promotes

the recruitment of circulating leukocytes into the inflammatory joint, resulting in angiogenesis to provide nutrition and oxygen to the hypertrophic joint. Fibroblast-like synoviocytes (FLS) in the synovium intima adopt an invasive character, promoting extracellular matrix invasion and aggravating joint damage. [5]

- FLS and immune cells in RA

Previous studies on RA have discovered that in occurrence and progression of disease there is great role of immune cells. Stromal cells forming structural and functional framework of organs are considered to have ability to trigger immune responses.

Various monocytes and macrophages activates osteoclasts and fibroblasts interact directly with the dendritic cells, pre-osteoclasts etc. Due to cytokine attachment to the receptors genes are activated which are futher responsible for inflammation and tissue damage. (R5)

2.1.2 Therapy in RA and associated risks

The main objective of RA therapy includes reduction of pain and inflammation, slow joint deterioration, and to enhance physical function and overall quality of life. NSAIDs, immunosuppressive glucocorticoids, and disease-modifying anti-rheumatic medications (DMARDs) are now used for treating of rheumatoid arthritis. DMARDs are further classified as traditional, biologic, and small molecule DMARDs (smDMARD).[13]

NSAIDs, such as diclofenac and ibuprofen, are used to treat cases in which pain and stiffness reduction is required, this therapy cannot slow down disease development. Glucocorticoids are frequently used in combination with other therapeutic options in RA treatment; however their associated toxicity and side effects are very significant

Glucocorticoids can be used as a supplement to standard DMARDs to enhance disease activity. Previous studies revealed that combining traditional DMARD therapy with prednisone improved disease activity and decreased tiredness in RA patients compared to DMARD therapy alone.

The first-line agents such as methotrexate (MTX), leflunomide (LEF) etc. are usually accompanied with a wide range of side effects such as hepatotoxicity, mucositis, and leukopenia, resulting in an unsatisfactory response.[4]

SSZ has been associated with side effects such as CNS toxicity and drug-induced systemic lupus erythematosus

Side effects of HCQ include permanent toxic retinopathy, which causes retinal thinning and photoreceptor impairment.

Because of poor responses and significant side effects associated with standard DMARDs, biological DMARDs (bDMARDs) have emerged as a more refined and targeted type of immunosuppressant. They are used when synthetic DMARDs do not produce sufficient results. Currently authorized bDMARDs for RA include drugs that neutralize TNF-α or IL-6, and deplete B cells. The major side effects of bDMARDs include an increased risk of common and severe infections. These infections include UTIs, allergic responses, and the recurrence of TB, and herpes zoster.

Another established therapy for RA includes JAK inhibitors, which will be discussed further. [14]

2.2 Understanding JAK/STAT Pathway

2.2.1 JAK/STAT Signalling Pathway

When cytokines or growth factors bind to the appropriate transmembrane receptors, signalling begins. This contact puts receptor-bound JAKs into close proximity, allowing for trans-activation and cause conformational changes separating their kinase domains from inhibitory pseudokinase regions. Upon activation, JAKs further causes phosphorylation of latent STAT monomers, causing their dimerization, go to the nucleus, and bind to DNA. (7) The Janus kinases (JAKs), which comprise JAK1, JAK2, JAK3, and TYK2, are tyrosine kinases of nonreceptor protein type. Unusual activation of the JAK/STAT signaling system, caused by mutations in JAK or constitutive TYK2 signaling, is crucial in the generation of abnormal hematopoietic stem cells, autoimmune disorders, and some immunodeficiency syndromes. As a result, reducing JAK activation has been demonstrated to influence T-cell, and dendritic cell function, all significant to the development and progression of autoimmune diseases. It is commonly known that STATs may bind directly to DNA and function as classic transcription factors (TFs). [7]

This pathway is usually activated upon a ligand or cytokine serving as an extracellular signal by interacting to a receptor of the cell membrane. This interaction causes a structural or conformational change, causing activation of the implicated JAK isoforms, which can form homodimers or heterodimers. JAK auto-phosphorylation produces a docking site for the STAT protein, which is then phosphorylated upon binding. The JAKs aid in the translocation of STAT proteins into the nucleus, where gene expression begins, followed by protein synthesis, now we have discussed a simplified description of the functional consequences of the JAK/STAT signaling pathway. Various JAKs and STATs combinations form complex multimers, causing a variety of biochemical and biophysiological consequences both inside cells and across the body. Deficiencies in some JAKs, or genetic gain-of-function mutations in others, form the clinical foundation for a wide range of illnesses.[2]

2.2.2 JAK2 as a treatment target

JAK2 activates STAT3 and STAT5, and signals through many receptors, including IL-6R, and IFN-γ R2. JAK2 has been associated to a variety of illnesses, including blood problems,cancer, and autoimmune conditions. In RA patients, JAK2 expression in synovial tissue is considerably higher than in healthy persons. Similar findings have been reported in previous studies conducted on animal models.

In animal arthritic models, a highly selective JAK2 inhibitor dramatically lowered cytokine levels (IFN-γ, IL-12, and TNF-α) and serum IL-2, IL-12, and p-Stat 3 in synovial fluid. These data suggest that JAK2 plays a role in RA pathogenesis and that decreasing JAK2 can cure RA by lowering cytokine production and activation of T and B cells. Furthermore, receptors phosphorylated by JAKs can bind PI3K, initiating the PI3K-AKT pathway.[8]

2.2.3 Approved JAK Inhibitors

As of now four JAK inhibitors have been authorized in Japan, with filgotinib now undergoing the approval process. Tofacitinib, baricitinib, and peficitinib have been introduced. There are four kinds of JAKs, and normally, two of them after connecting with cytokine receptors to send signals. The selectivity of the five JAK inhibitors described above varies to some extent. A wide range of cytokines are repressed depending upon the JAK targeted from all four, hence the outcomes may vary. The effectiveness and disadvantages observed in clinical practice may vary

across the five JAK inhibitors. However, the relationship between variances in JAK inhibitory activity and clinical outcomes is not as strong as expected. Comparative effectiveness and safety of JAK inhibitors (tofacitinib, baricitinib, upadacitinib, filgotinib) indicate that baricitinib and upadacitinib may be more efficacious but have a higher risk of severe infections and herpes zoster. But is is still unknown that the varied specificities of JAK inhibitors result in variations in clinical effectiveness and safety or not.

Peficitinib is unusual in that it largely inhibits JAK3, whereas the others primarily inhibit JAK1. Tofacitinib, peficitinib, and upadacitinib do not impact TYK2, but baricitinib and filgotinib do not inhibit JAK3. Baricitinib also inhibits JAK2, unlike the others. Furthermore, because JAK inhibitors work in pairs, these profiles may be insufficient to adequately define their action, further studies are needed to determine the optimal use of different JAK inhibitors. [2]

A few of popularly known approved JAK inhibitors are given below:

- **Tofacitinib**

Tofacitinib is a non-selective, first-generation JAK inhibitor that predominantly treats RA, PsA, and ulcerative colitis. It inhibits JAK1, JAK2, JAK3, and, to a lesser degree, TYK2.. Tofacitinib requires a dose of 5 mg twice day; once administered, it is quickly absorbed and reaches maximal concentration (Tmax) in around 0.5 to 1.0 hours. The drug's pharmacokinetics are dosage-proportional, with a steady state 24 to 48 hours after the initial dose. Tt's half-life is 3.2 hours. [15]

- **Baricitinib**

Baricitinib belongs to the first generation of Janus kinase inhibitors (JAKi). Currently, it is only approved for the treatment of moderate to very severe active rheumatoid arthritis (RA) in adult patients who do not react well to or cannot take one or more conventional disease-modifying antirheumatic medicines (cDMARDs). It has the chemical structure of a pyrrolopyrimidine, causing its insolubility in water but somewhat soluble in hydrochloric acid. Baricitinib prevents the activation of both JAK1 and JAK2 molecules. As a result, it inhibits interleukin-6 expression in a dose-dependent manner. In healthy subjects given a daily dosage, inhibition peak is attained two hours after treatment and lasts for 24 hours. Another important impact of baricitinib

is the prevention of osteoclastogenesis in vitro which reduces subchondral bone erosions by down-regulating the receptor activator of nuclear factor-B ligand in osteoblasts. [16]

2.2.4 Side Effects Profile of Approved JAK Inhibitors

Infections are the most prevalent side effects of JAK inhibitor therapy in RA patients. While the prevalence of common diseases such as upper respiratory tract infections, lower respiratory tract infections, and urinary tract infections is higher than in the general population, it is comparable to that seen with biologic DMARDs. Notably, studies have shown that tofacitinib has a reduced risk of infection than TNF inhibitors, such as tocilizumab. JAK inhibitors also had a somewhat reduced risk of serious infection as compared with bDMARDs. Also, tuberculosis develops at a much higher rate patient under this traetment. As a result, quantiferon testing is suggested as part of the screening process before beginning JAK inhibitor treatment.[17]

- **Herpes Zoster Infection**

Herpes Zoster (HZV) infection is more frequent in RA patients than in the general population, owing to variables like as age and immunosuppression associated with prolonged corticosteroid usage. However, the introduction of medications targeting the JAK pathway generated worries of a possibly large increase in the risk of HZV infection. Data from tofacitinib randomized controlled trials (RCTs) show that the probability of HZV infection is 1.5 to 2 times greater than in the general RA population and higher than in patients using biologic DMARDs. While some of this increased risk may be due to regional variances or ethnicity, greater rates have been recorded in Asia and India compared to Western Europe and North America, where infection rates are substantially lower. This disparity may be due to the downregulation of interferons and IL-15, both of which are important in viral eradication.[18]

However, it is encouraging to note that multi-dermatomal and disseminated herpes zoster cases were infrequent, and there were no reports of visceral illness or mortality linked with tofacitinib therapy.

- **Cytopenias**

Cytopenias, can develop with any JAK inhibitor used to treat RA. Interestingly, the cytopenic effect is the mechanism of action for Ruxolitinib, a JAK inhibitor used to treat myeloproliferative diseases. While JAK inhibitors may potentially have a protective effect

against lymphomas and leukemias by inhibiting the JAK2 pathway, clinicians must stay watchful regarding cytopenia, especially neutropenia and lymphopenia, which increase the risk of infection.[19]

It's worth mentioning that, while cytopenia is a usual side effect of JAK inhibitors, there are several explanations for anaemia in RA. Tofacitinib, for example, may modestly boost hemoglobin levels because it has a lower inhibitory impact on JAK2, which is crucial for erythropoietin signalling.

Furthermore, at two weeks, temporary and mild platelet increase was found with baricitinib, which might be attributed to poor dose. However, no adverse effects from this rise have been documented.

- **Thrombosis**

Post-marketing safety surveillance of pharmaceuticals has demonstrated an elevated risk of pulmonary embolism (PE) and death associated with tofacitinib at the daily dose in RA. While it is also important to consider that this dosage is not recommended for RA therapy, it is authorized for ulcerative colitis. This elevated risk of PE gradually revealed, in contrast to the first comforting results from pooled analyses of randomized controlled trials (RCTs).

Interestingly, the mechanism of action of JAK inhibitors does not immediately signal this concern because these medications frequently produce thrombocytopenia rather than thrombocytosis. However, the developing understanding emphasizes the significance for rheumatologists to assess a patient's history of past venous thromboembolism before starting treatment with a JAK inhibitor.

2.3 Potential of natural agents against arthritis

The previous sections have provided extensive scientific information on the toxic symptoms and how the use of conventional, biological, and small molecule JAK/STAT inhibitors in RA therapy has been linked to significant infections. Furthermore, the high price of thes. Due to these negative consequences, scientists as well as RA patients are continually looking for alternative therapies, which are frequently discovered in nature and have increasingly been the focus of the pharmaceutical sector. Plant-derived medications capable of controlling the generation of pro-

inflammatory signals have showed promising outcomes in arthritis treatment. Flavonoids, quinones, alkaloids, anthocyanins, and anthoxanthins are all anti-inflammatory compounds. Various herbs, including curcuma longa, quercetin, and andrographolide, have been demonstrated in studies to have anti-arthritic action, which may help relieve inflammation and discomfort in RA patients. e medications contributes to limited availability, particularly in underdeveloped nations.[9]

2.3.1 Quercetin

Quercetin, a flavonoid, is renowned for its anti-rheumatoid arthritis (RA) properties. In studies in which quercetin was administered orally to rats with collagen-induced arthritis at a dosage of 150 mg/mL, it reduced IL-17A and IL-21 levels. Quercetin is known to lower TNF-α levels. A randomized controlled trial of 100 women revealed that taking quercetin daily for eight weeks reduced pain and TNF-α level. In rats, hesperidin significantly decreased joint damage and serum TNF-α levels compared to the control group, demonstrating preventative capabilities against RA.

Quercetin may decrease autoimmune arthritis by reducing neutrophils' inflammatory activity. Arthritic mice treated with quercetin showed substantial decreases in ankle diameters and arthritic scores. Quercetin has been shown in studies to decrease neutrophil infiltration both in vivo and in vitro, enhance neutrophil death in response to LPS stimulation, and inhibit PMA-induced NET formation in an autophagy-dependent way. This suggests that quercetin's anti-arthritic actions are enhanced by its reduction of neutrophil activity. Neutrophils account for 60% of leukocytes in peripheral blood and are critical to the etiology of rheumatoid arthritis (RA). Abnormal neutrophil accumulation in tissues causes uncontrolled inflammation and tissue damage in RA. Neutrophils in joints produce and emit inflammatory cytokines such as IFN-γ and IL-6. In mouse studies, quercetin lowered neutrophil infiltration and inflammatory cytokine levels. Thus, quercetin can decrease neutrophil invasion and relieve inflammation. [20]

2.3.2 Andrographolide

Andrographis paniculata is a traditional herbal remedy used in Taiwan, China, India, and other Southeast Asian nations to treat inflammation-related illnesses. Andrographolide (AD), a diterpenoid, is well-known as an active pharmaceutical component of A. paniculata. AD is known for inhibiting tumor metastasis, oxidative stress, and inflammatory reactions, as well as

having antibacterial properties. Recently, AD and its derivatives have received a lot of interest for their anti-inflammatory and antioxidant properties. However, the efficacy of AD in treating human rheumatoid arthritis (RA) has yet to be proved. The aim of this study is to look at the impact of Alzheimer's disease on the therapy of arthritis. [21]

2.3.3 Ellagic Acid

Ellagic acid (EA) is a phenolic molecule present in many nuts and fruits. The possible anti-inflammatory qualities of EA have recently sparked increased interest in the substance. EA lowers inflammation via modulating NF-kB activity and inhibiting IL-1β-induced nuclear translocation of p65 and p50. Several investigations have shown that EA regulates both pro- and anti-inflammatory cytokine productions. EA reduces IL-13 and TNF-α production from stimulated human peripheral blood mononuclear cells, whereas IL-4 production remains steady. EA dramatically reduces mRNA expression and cardiac levels of IL-1, TNF-α, and MCP-1. EA reduces IL-6, TNF-α, and MCP-1 levels in the kidney and down-regulates their mRNA expression. In mouse models, EA ingestion significantly reduces kidney levels of IL-1β, IL-6, and TNF-α. Applying EA topically decreases pro-inflammatory cytokines IL-1β and IL-6 and prevents inflammatory macrophages from infiltrating UV-B-exposed SKH-1 hairless mice's skin. In stomach ulcerated rats, EA therapy reduces pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and increases anti-inflammatory cytokines (IL-4 and IL-10) [25]. EA effectively reduces neutrophil infiltration, TNF-α, and IL-1β levels in AIA. Furthermore, EA therapy decreases IL-6 levels in bronchoalveolar lavage fluid while increasing IL-10.

Overall, EA has been demonstrated to reduce pro-inflammatory mediators while increasing anti-inflammatory cytokine production, indicating that it may have a role in the prevention and/or treatment of rheumatoid arthritis.[22]

2.3.4 Curcumin (Curcuma longa)

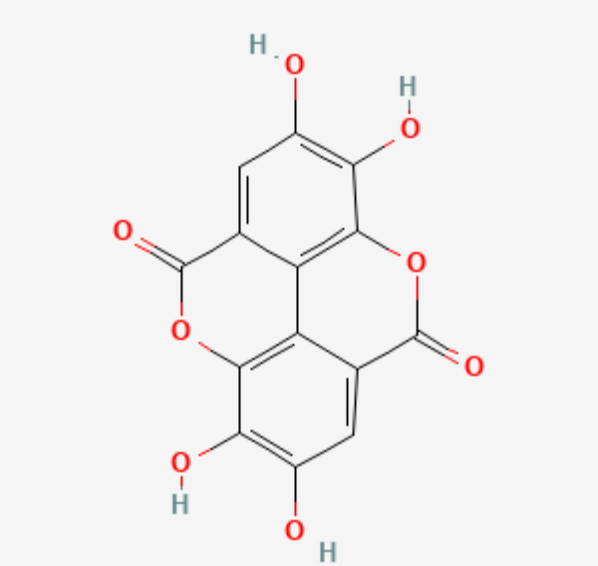
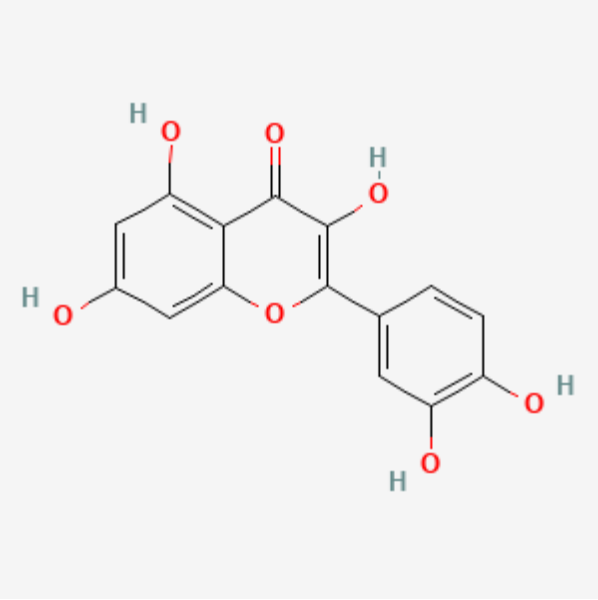
Turmeric, formally known as Curcuma longa, is a fragrant herbaceous plant native to Southeast Asia that is classed in the ginger family; India is a major producer, user, and exporter of turmeric on a worldwide scale. The bright orange powder derived from the turmeric rhizome has long been a staple of Ayurvedic cuisine and holistic health, acting as a valued medicinal plant. Curcumin, the primary bioactive component of turmeric, has strong pharmacological actions

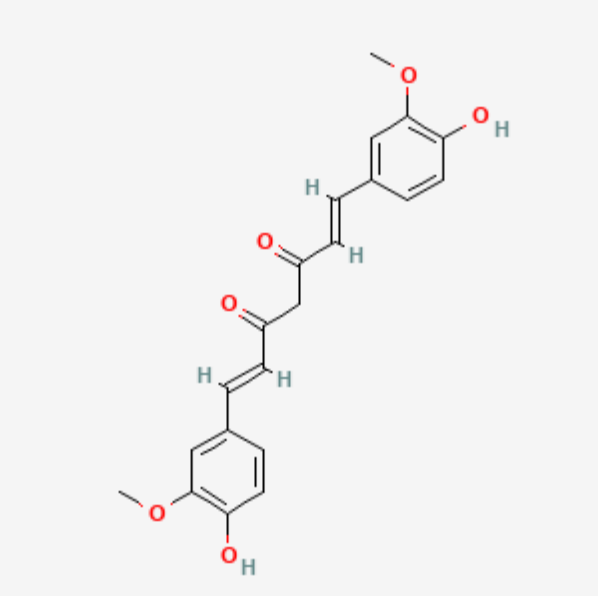
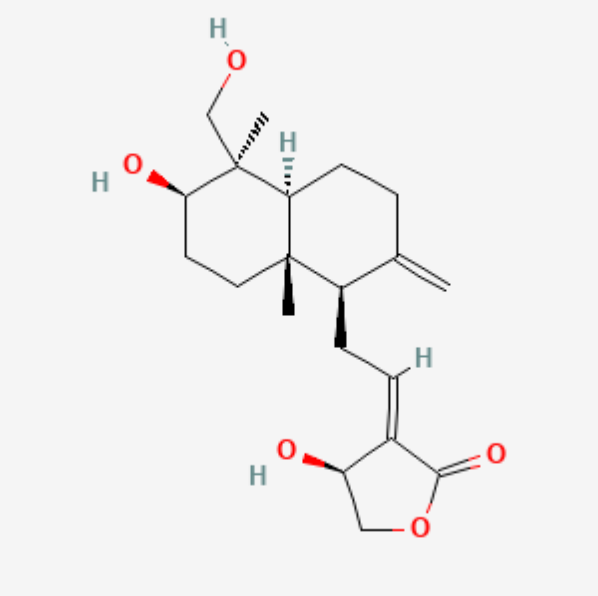
such as antioxidant, anti-inflammatory, anti-angiogenic, and anti-tumor activities, with few side effects. Numerous clinical studies are now being conducted to investigate the health benefits of curcumin, with a focus on cancer, obesity-related metabolic diseases, and neurology. Furthermore, several studies have shown that curcumin and curcuminoids found in turmeric provide significant protection against a variety of chronic conditions by inhibiting inflammatory responses, lowering blood cholesterol levels, and improving blood sugar management. Curcumin has showed promising results in the treatment of Crohn's disease, colorectal and prostate cancer, ulcerative colitis, and autoimmune illnesses such rheumatoid arthritis and inflammatory bowel diseases. As a result, it can be claimed that curcumin is helpful not only in autoimmune illnesses, but also as an anti-inflammatory and anticancer drug.[23]

Curcumin's anti-arthritic potential has already been established. The clinical characteristics of RA, such as cartilage degradation, are caused by neutral matrix MMPs, although curcumin has been demonstrated to decrease the increase of MMP mRNA associated with arthritis. his polyphenol also suppresses the production of TNF-a-induced MMP-13 in primary chondrocytes. Curcumin has been shown in studies to suppress neutrophil activation, angiogenesis, and collagenase expression, indicating that it may have therapeutic promise in arthritis.[24] Furthermore, Curcumin has been demonstrated to enhance the growth inhibitory and pro-apoptotic effects of the COX-2 inhibitor celecoxib in osteoarthritis synovial adherent cells. A recent study discovered that curcumin lowers NF-kB activation, inhibiting COX-2 and MMP-9 synthesis in human articular chondrocytes. Aside from these findings, in vivo research has shown curcumin's usefulness against arthritis. For example, curcumin treatment when taken orally has been demonstrated to diminish elevated levels of the glycoprotein Gp A72 and paw inflammation in arthritic rats. [25]

Curcuma longa's many components are utilized therapeutically and incorporated into the Ayurvedic medical system.[26] As a result, this study will use the PyRx program to examine several natural phytochemical ingredients as Janus kinase inhibitors. The pharmacological characteristics of these compounds were evaluated, and a target prediction assay was used to determine the best-fitting protein-ligand combination.

Table 1- 2-D Structures of Phytochemical Inhibitors of JAK2

Phytochemical	Structure of Phytochemical
Ellagic Acid	
Quercetin	

Curcumin	
Andrographolide	

CHAPTER 3

METHODOLOGY

3.1 Software Used

3.1.1 PDB

Brookhaven National Laboratory and the Cambridge Crystallographic Data Centre collaborated to create the Protein Data Bank (PDB). In 1999, the Consortium overseeing PDB Research Collaboratory of Structural Bioinformatics (RCSB) was created, and it is today the most visited wwPDB Web site. On a monthly basis, over 100,000 visitors register on this website, generating approximately 500 GB of data flow. It is a well-designed Web interface with a range of free user-friendly 3D viewers accessible, allowing it to be used by educational courses as an information source. PDB data repositories rely heavily on its deposition techniques. These tools are designed with a user-friendly, well-defined interface, as well as data processing and validation in mind. The fundamental purpose of PDB design was to incorporate sophisticated algorithms that would provide a seamless and error-free experience. [27]

3.1.2 BIOVIA Discovery Studio Visualizer

BIOVIA Discovery Studio, created by Dassault Systèmes, is a versatile and powerful software suite tailored for computational chemistry, molecular 19ehaviour, and bioinformatics. It serves a broad spectrum of scientific research needs in drug discovery, materials science, and life sciences. Among its extensive features are molecular docking and molecular dynamics, which help researchers predict how molecules interact and behave over time, as well as QM/MM simulations that combine quantum mechanical and molecular mechanical calculations for detailed analysis of complex systems. In protein 19ehaviour, Discovery Studio excels with tools for homology 19ehaviour, allowing the prediction of 3D structures based on known related proteins, and protein-protein docking to explore interactions and binding affinities between proteins. For drug design, it supports both structure-based and ligand-based approaches, offering capabilities such as pharmacophore 19ehaviour to identify key features for biological activity. The suite also includes robust cheminformatics tools for screening chemical libraries and developing QSAR/QSPR models to predict the properties of new compounds. Bioinformatics

functionalities cover sequence analysis, as well as genomics and proteomics, facilitating the analysis and interpretation of large-scale biological data. Additionally, it offers materials 20ehaviour for simulating and understanding the properties and 20ehaviour of materials at the molecular level. Enhanced by advanced 3D visualization tools and an intuitive graphical user interface, BIOVIA Discovery Studio is designed to streamline and accelerate the research process, making it an invaluable asset for scientists and researchers in various fields.[10]

3.1.3 PyRx Docking Software

PyRx is an open-source software application that has become an indispensable tool in the fields of computational chemistry and drug discovery due to its capabilities in virtual screening and molecular docking. This software is designed to facilitate the prediction of how small molecules, such as potential drug candidates, interact with target proteins, which is a critical step in the drug development process. One of the standout features of PyRx is its user-friendly graphical user interface (GUI), which makes it accessible to both experienced researchers and those who may not have extensive backgrounds in computational chemistry or bioinformatics. The intuitive interface integrates various computational tools and provides visualizations that streamline the analysis and interpretation of complex molecular interactions.

PyRx integrates popular tools like AutoDock Vina and Open Babel. AutoDock Vina is renowned for its accuracy and speed in predicting the preferred orientations of small molecules when they bind to their target proteins. This feature is particularly valuable in virtual screening, where researchers need to sift through vast libraries of compounds to identify promising candidates that can be further investigated and optimized. Open Babel, another key component of PyRx, serves as a chemical toolbox that enables the interconversion of different file formats, which is essential for managing and manipulating chemical data. Additionally, Open Babel provides various cheminformatics functionalities that enhance the overall capabilities of PyRx.

The molecular visualization tools within PyRx are robust and highly interactive, allowing researchers to view and manipulate molecular structures and docking results in three dimensions. This visual aspect is crucial for understanding the intricacies of molecular interactions and for refining drug candidates based on their binding affinities and conformations. By visualizing these interactions, researchers can gain insights into the structural features that contribute to the

binding efficacy of a compound, which can inform subsequent modifications to improve its drug-like properties.

Another significant advantage of PyRx is its cross-platform compatibility, making it accessible to users operating on Windows, macOS, and Linux systems. This flexibility ensures that a wide range of researchers can utilize the software regardless of their preferred operating environment. PyRx's open-source nature supports a thriving community of users and developers that contribute to its continuous growth and enhancement. PyRx's community-driven approach ensures that it is always changing to meet the demands of its users, with frequent updates and upgrades made accessible.[26]

In the context of drug discovery, PyRx's ability to perform high-throughput virtual screening efficiently can save considerable time and resources. Traditional methods of drug discovery often involve labor-intensive and costly experimental screening processes. By using PyRx to conduct virtual screenings, researchers can quickly narrow down the list of potential drug candidates to those that are most likely to succeed, thereby focusing their experimental efforts on the most promising compounds. This not only accelerates the drug discovery process but also reduces the overall costs associated with developing new therapeutics.

3.2 Molecular Docking

Molecular docking is one of the most important techniques under structural molecular biology; it has main applications under computer-aided drug creation. Prediction of most common binding mode(s) of a protein with known 3-D structure is the primary goal of ligand-protein docking. Docking algorithms scan high-dimensional areas and evaluate likely docking by providing a docking score through a scoring system. This technique therefore, can be used to screen large chemical libraries digitally, to further analyse the findings to provide suggestions about ligand behaviour. It is usually performed between a target protein and small molecule ligand. Molecular docking has a wide range of applications in drug discovery, which includes structure-activity relationship studies, virtual screening for potential leads, generating binding hypotheses to aid in mutagenesis predictions, assisting in fitting substrates and inhibitors to electron density in X-ray crystallography, studying chemical mechanisms, and designing combinatorial libraries of the target, which is extremely useful for lead optimization.[26]

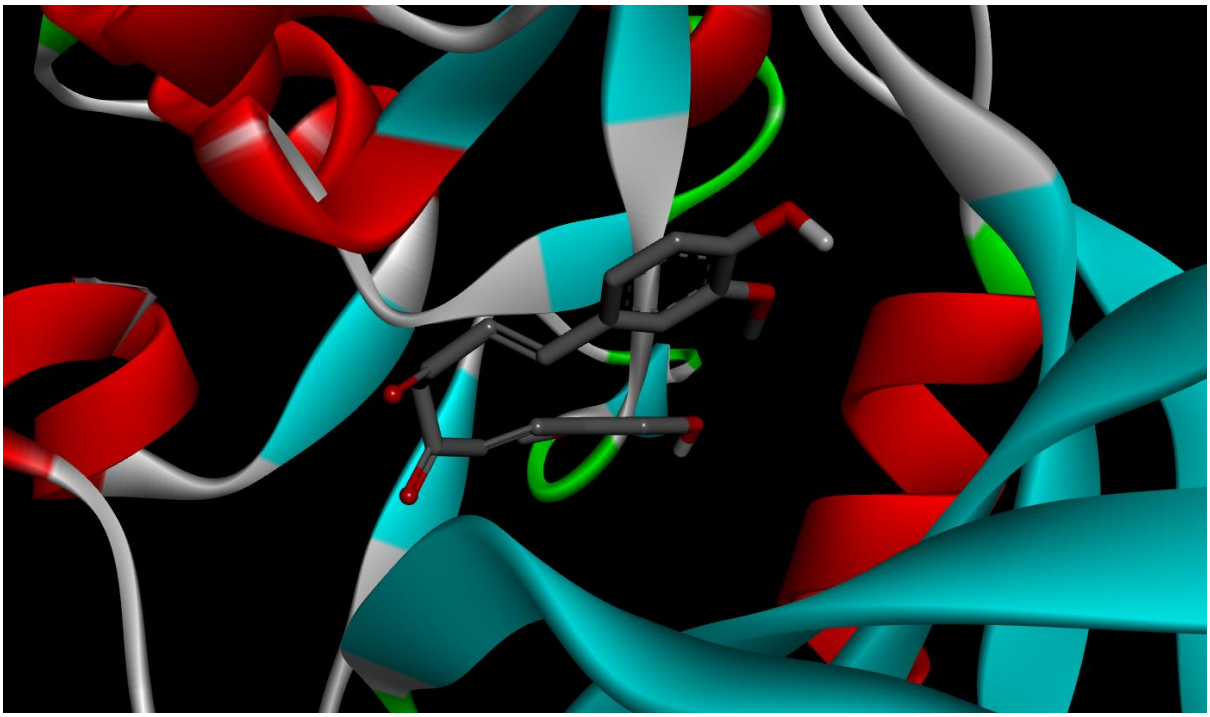


Fig 1- Molecular Docking

3.2.1 Target Selection and Preparation

The target structure for molecular docking should be experimentally established, often using X-ray crystallography or nuclear magnetic resonance. Homology modelling can be utilized to carry out docking, but their quality and accuracy have a substantial influence on the reliability of the results. In some cases, the medically relevant form of the target structure is a multimer that necessitates the inclusion of symmetry-related molecules. For example, the online database Binding MOAD provides target structures as biological units for docking studies.

3.2.2 Ligand Selection and Preparation

The specific goal of this step is to determine the ligands used for docking. This process is carried out to minimize the number of molecules docked, for filtration of ligands crude filters can be applied for example net charge, polar surface area, solubility, commercial availability, price-per-compound, and molecular weight, further lead optimization criteria include similarity thresholds, synthetic accessibility, properties relating to absorption, distribution, metabolism, excretion, and

pharmacophores. For performing targeted lead optimization, a customized library of analogues which are linked with the lead compound(s) is usually created for docking, directing, and prioritizing medicinal chemistry activities. Except for ring conformations, most docking tools include a flexible approach to ligands. As the number of rotatable bonds in a ligand grows, so does the difficulty and time required for docking. These issues are due to growth of search space exponentially with multiple torsions. It is much more challenging with torsion tree with more branches as compared to a much more linear torsion tree. Amide, urea, carbamates etc. contain conjugated bonds which require a much more in depth monitoring.

3.2.3 Docking

Molecular docking is the computer exploration of a search space defined by the method's molecular representation, followed by a ranking of potential solutions to determine the best binding mode. This strategy requires a search technique along with a grading system. Search techniques are usually of two types: systematic and stochastic. The search output in systematic procedures is predictable, but the response quality is controlled by how finely the search space is sampled. Stochastic techniques, on the other hand, incorporate some degree of unpredictability, causing the outcome to shift. Systematic search strategies are usually utilized in stiff protein-rigid protein docking.

Numerous studies have investigated functions of scoring in molecular docking. These functions can be either knowledge based, or molecular mechanics based. Docking algorithms also employ a docking function prior and another after docking; this is performed to re-rank the results. Though, this approach of scoring in retrospective manner has no major impact on the primary efficacy or accuracy.

3.2.4 Evaluating Docking Results

Docking data should always analysed in terms of chemical complementation between ligand and protein. Important aspects to be considered includes fulfilment of all possible hydrogen bond donor and acceptors in the ligand, along with making sure that whether there is interaction of charged groups and oppositely charged side chains in receptor or are buried in hydrophobic pockets, along with interaction of hydrophobic groups being properly positioned within hydrophobic pockets in the receptor.[10]

3.3 Procedure

i. **Data collection: -** The compilation of selected natural compounds was derived from scientific literature, which highlighted the potential of these compounds for suppressing the progression of rheumatoid arthritis. The PubChem database was used to obtain the desired SMILES (Simplified Molecular-Input Line-Entry System) structures for the selected natural compounds. SMILES is a widely used chemical notation system that provides a compact representation of the molecular structure, which is essential for computational analysis and molecular docking studies.

ii. **Preparation of target and ligand:** - The protein structure utilized for the docking study was obtained from the **Protein Data Bank (PDB) entry 3TJC**, which contained the A chain with a sequence length of 575 amino acids. The three-dimensional structure of the target protein, JAK2, was prepared using **Discovery Studio** molecular visualization software. This preparation process involved the removal of any water molecules and ligands present. After the necessary modifications, the final prepared structure of the JAK2 protein was saved in the standard PDB file format, suitable for use in the subsequent docking simulations. For the ligand (compound) preparation, the initial structures in SDF format were converted to the MOL2 file format using the **Open Babel** software. This conversion step ensures compatibility with the docking software and facilitates the docking process. The transformed ligand structures in MOL2 format were then stored for further use in the molecular docking analysis.

iii. **Molecular docking using PyRx: -** The protein JAK2 (PDB ID: 3TJC) was opened in the PyRx virtual screening tool as the starting protein structure in pdbqt format. All ligands were selected and automatically converted to pdbqt format. After selecting both the protein and ligands, the grid box appeared automatically, and the center of the target site was assigned. Docking was performed using AutoDock Vina. In this study, the phytochemical ligands andrographolide, curcumin, ellagic acid, and quercetin were compared with the well-known and approved JAK inhibitor tofacitinib. The best free energy of binding values were obtained using the PyRx virtual screening tool.

CHAPTER 4

RESULTS

4.1 Docking Results

The binding mechanisms and interactions between phytochemicals and the JAK2 protein are predicted using molecular docking simulations. The phytochemical ligand's position within the protein's active region as well as its binding to particular binding sites on JAK2 are depicted in the simulations. Clarifying the possible inhibitory mechanisms of various inhibitors on JAk2 signalling pathways requires an understanding of the unique binding modes and orientations of ligands inside the JAK2 active site. Researchers can learn a great deal about the structure-activity correlations and direct future improvement of phytochemicals or their derivatives as possible JAK2 inhibitors by identifying the important amino acid residues involved in the binding interactions and the overall binding posture of inhibitor.

All phytochemicals satisfied the criteria i.e. ellagic acid with binding affinity -9.8, quercetin with binding affinity -8.9, curcumin with binding affinity -8.4, and andrographolide with binding affinity -7.6 which is lower than tofacitinib, an approved inhibitor of JAK. All phytochemicals proved to be more effective inhibitors than anticipated with tofacitinib having a binding affinity of -7.3.

4.2 Docking between JAK2 and Ellagic Acid

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
jak_2_5281855_uff_E=227.58	-9.8	0	0
jak_2_5281855_uff_E=227.58	-9.8	6.183	0.069
jak_2_5281855_uff_E=227.58	-9.5	5.08	1.085
jak_2_5281855_uff_E=227.58	-9.5	3.62	1.094
jak_2_5281855_uff_E=227.58	-9.3	3.567	1.377
jak_2_5281855_uff_E=227.58	-9.3	4.914	1.151
jak_2_5281855_uff_E=227.58	-9.2	5.635	1.287
jak_2_5281855_uff_E=227.58	-9.1	4.473	1.258
jak_2_5281855_uff_E=227.58	-8.9	4.811	1.416

Table 2- Evaluation of binding affinity between ellagic acid and JAK2.

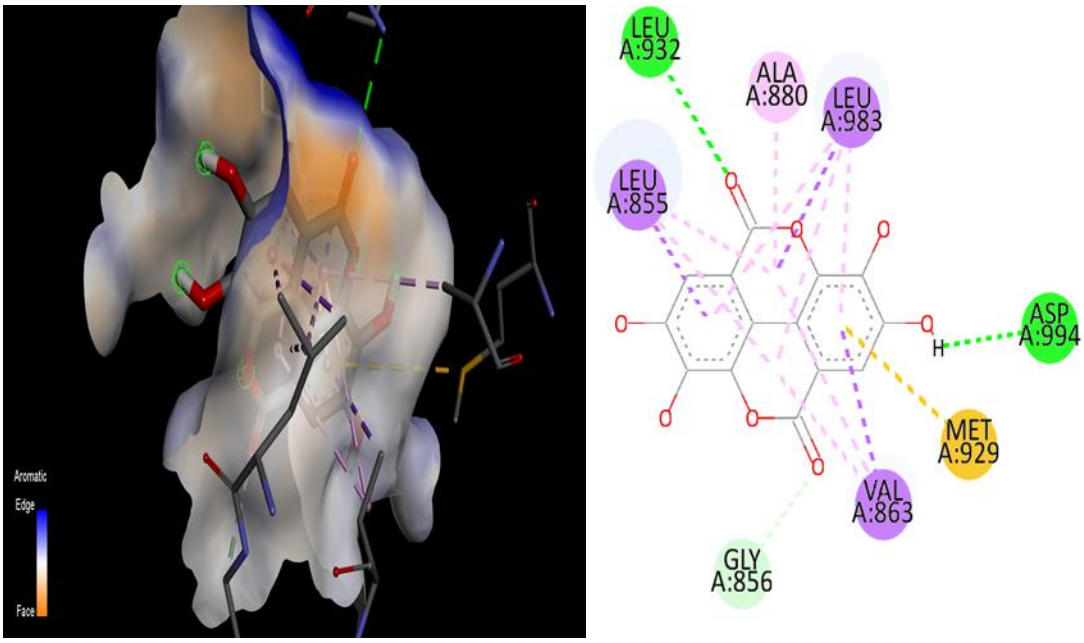


Fig 2- 3-D view of JAK2 docked with ellagic acid and visualization of target protein's amino acid.

4.3 Docking between JAK2 and Quercetin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
jak_2_5280343_uff_E=380.43	-8.9	0	0
jak_2_5280343_uff_E=380.43	-8.7	3.212	1.372
jak_2_5280343_uff_E=380.43	-8.3	1.615	1.223
jak_2_5280343_uff_E=380.43	-8.1	4.591	2.675
jak_2_5280343_uff_E=380.43	-7.9	7.262	1.688
jak_2_5280343_uff_E=380.43	-7.9	5.568	3.672
jak_2_5280343_uff_E=380.43	-7.7	7.27	1.811
jak_2_5280343_uff_E=380.43	-7.5	30.209	27.917
jak_2_5280343_uff_E=380.43	-7.4	29.607	28.523

Table 3- Evaluation of binding affinity between quercetin and JAK2.

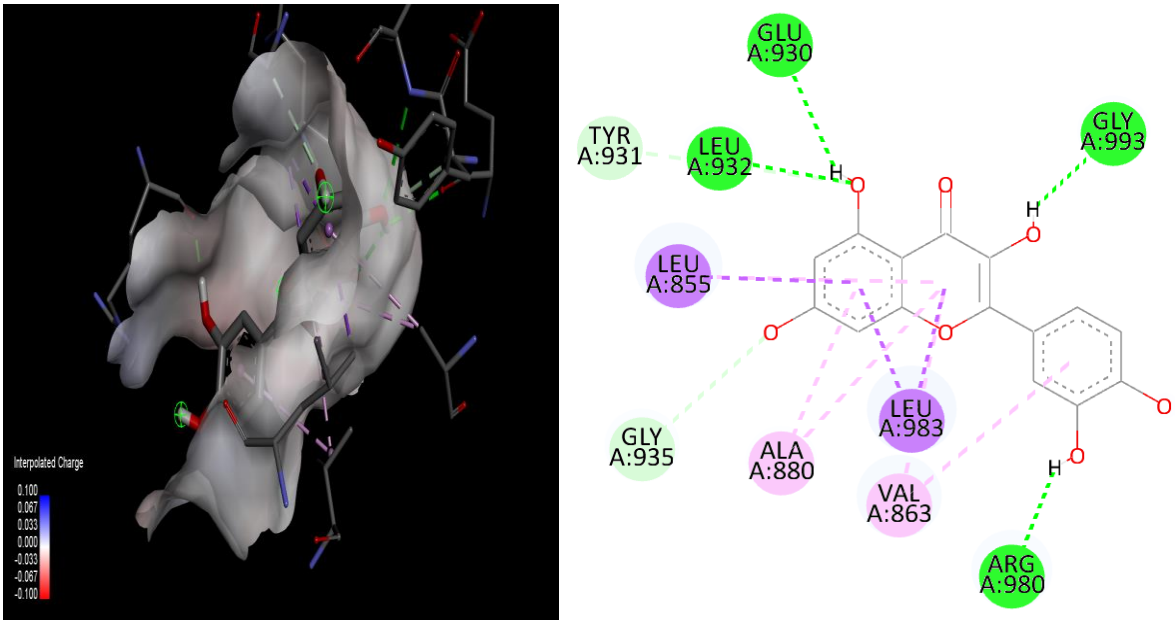


Fig 3- 3-D view of JAK2 docked with quercetin and visualization of target protein's amino acid.

4.4 **Docking between JAK2 and Curcumin**

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
JAK2_curcumin	-8.4	0	0
JAK2_curcumin	-8.4	1.757	0.71
JAK2_curcumin	-8.3	6.769	0.893
JAK2_curcumin	-8.1	6.988	0.668
JAK2_curcumin	-8.1	8.194	1.934
JAK2_curcumin	-8.1	8.273	1.896
JAK2_curcumin	-7.9	6.413	2.056
JAK2_curcumin	-7.8	7.285	2.043
JAK2_curcumin	-7.7	6.792	1.494

Table 4- Evaluation of binding affinity between curcumin and JAK2.

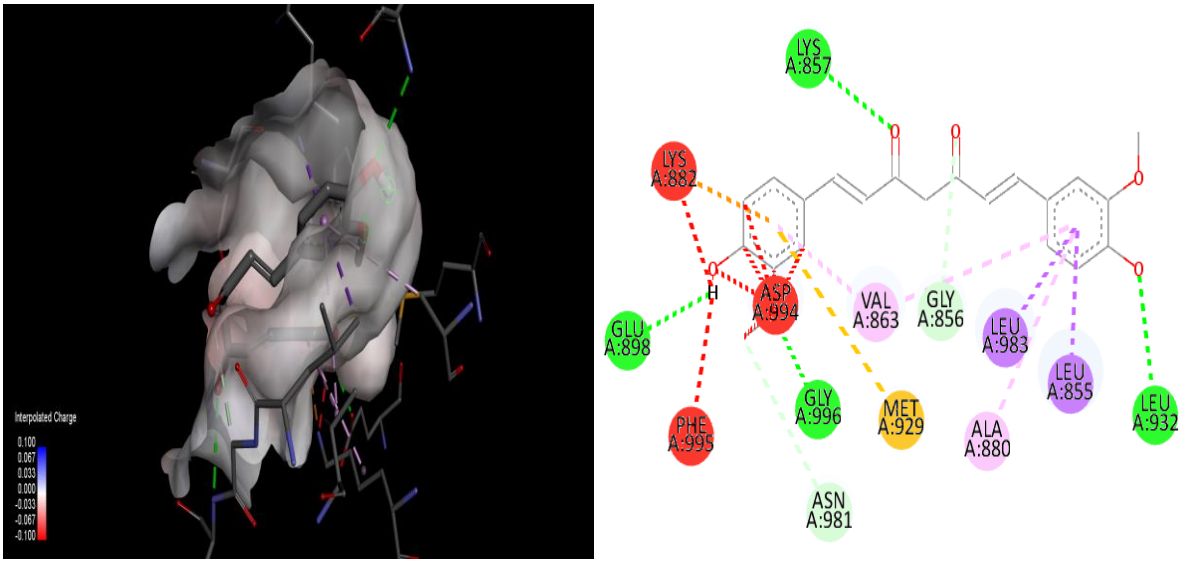


Fig 4- 3-D view of JAK2 docked with curcumin and visualization of target protein's amino acid.

4.5 Docking between JAK2 and Andrographolide

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
jak_2_5318517_uff_E=519.25	-7.6	0	0
jak_2_5318517_uff_E=519.25	-7.3	5.429	3.179
jak_2_5318517_uff_E=519.25	-7.3	33.075	31.032
jak_2_5318517_uff_E=519.25	-7.3	31.947	30.03
jak_2_5318517_uff_E=519.25	-7.1	3.627	2.018
jak_2_5318517_uff_E=519.25	-7.1	4.904	2.929
jak_2_5318517_uff_E=519.25	-7	29.573	27.626
jak_2_5318517_uff_E=519.25	-6.9	3.803	2.001
jak_2_5318517_uff_E=519.25	-6.9	1.897	1.432

Table 5- Evaluation of binding affinity between andrographolide and JAK2.

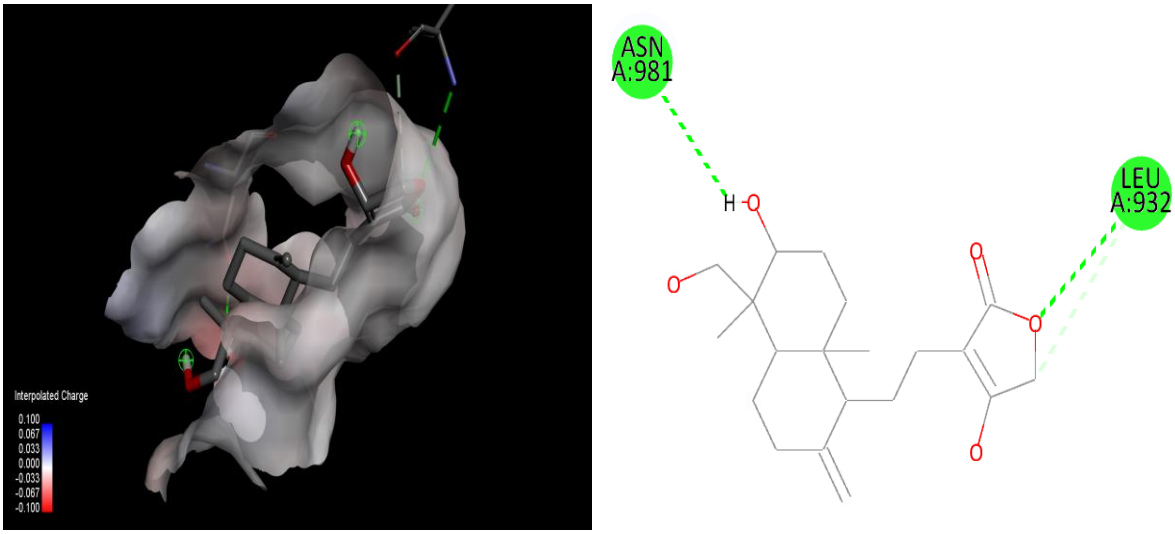


Fig 5- 3-D view of JAK2 docked with andrographolide and visualization of target protein's amino acid.

4.6 Docking between JAK2 and Tofacitinib (Control)

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
jak_2_9926791_uff_E=675.35	-7.3	0	0
jak_2_9926791_uff_E=675.35	-6.8	7.388	4.364
jak_2_9926791_uff_E=675.35	-6.5	6.06	3.517
jak_2_9926791_uff_E=675.35	-6.4	7.127	4.465
jak_2_9926791_uff_E=675.35	-6.2	6.385	5.101
jak_2_9926791_uff_E=675.35	-6.1	45.376	42.879
jak_2_9926791_uff_E=675.35	-6.1	18.818	16.505
jak_2_9926791_uff_E=675.35	-5.9	6.955	4.669
jak_2_9926791_uff_E=675.35	-5.7	7.959	4.485

Table 6- Evaluation of binding affinity between tofacitinib and JAK2.

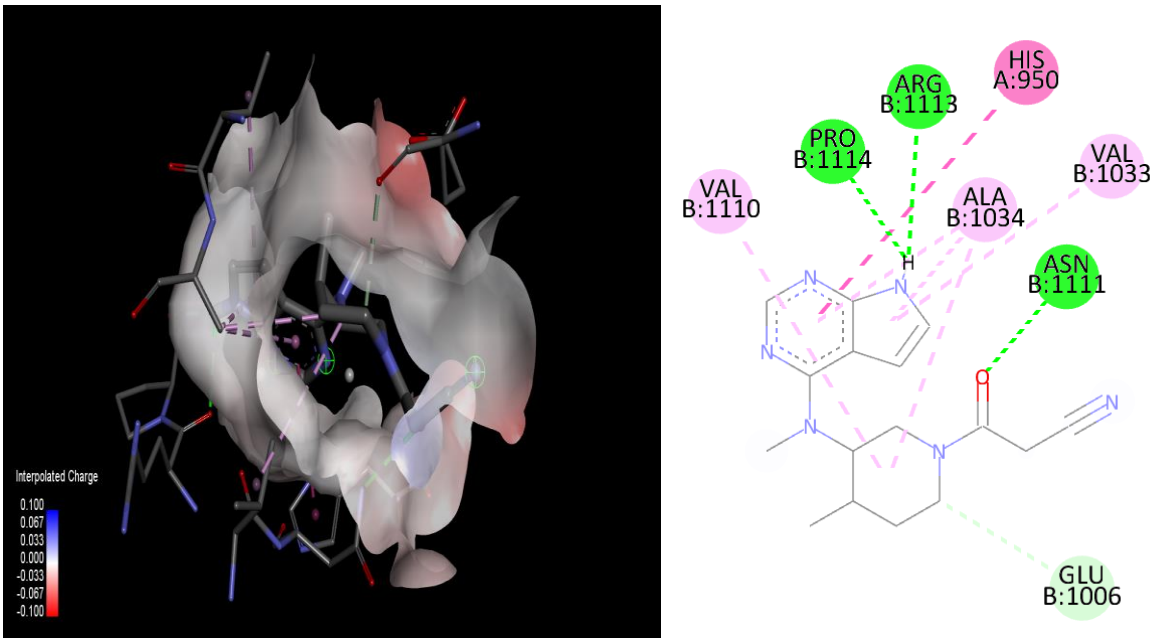


Fig 6- 3-D view of JAK2 docked with tofacitinib and visualization of target protein's amino acid.

4.7 Comparitive Analysis

Ligand	**Binding Affinity**
Ellagic Acid	-9.8
Quercetin	-8.9
Curcumin	-8.4
Andrographolide	-7.6
Tofacitinib	-7.3

Table 7- Comparitive analysis of all natural JAK2 inhibitors with control tofacitinib.

CHAPTER 5

DISCUSSION

The 3TJC protein was docked with the ligands that may have inhibitory properties against JAK2. Pyrrolopyridine is present in all ligands as the common scaffold. Tofacitinib, a JAK2 inhibitor licensed by the FDA, is the control ligand used. Tables 2 through 6 present the computed binding affinities. It is evident that most ligands docked using Autodock Vina had reasonable binding affinity values, which is shown by the negative value.

The phytochemicals' negative binding affinities have been shown to be rather high. The phytochemical with the highest negative binding affinity among all of them is ellagic acid, a phenolic component found in a variety of fruits and nuts. Fig. 2 displays its two-dimensional interaction diagram. With Asp994, Leu932 it interacts through hydrogen bonds. Additionally, the docking result revealed one pi-alkyl contacts with Ala880, and two pi-sigma interactions with Leu 983 and Val863. Additionally, Met929 displayed Pi-sulphur interaction.

The study revealed that the phytochemicals quercetin, andrographolide, and curcumin exhibit a high affinity for the target protein due to their high negative binding affinity. The ability of quercetin, andrographolide, and curcumin to cause cancer has already been reported.

Chemically speaking, quercetin is 3,30,40,5,7-pentahydroxyflavone(C15H10O7) is a polyphenolic flavonoid that occurs naturally and is frequently found in a variety of fruits and vegetables, including onions, dill, cilantro, capers, lovage, and berries like lingonberries, choke berries, and cranberries. Quercetin has anti-inflammatory and anti-cancer properties. It has been demonstrated that the diterpenoid lactone andrographolide, which is isolated from Andrographis paniculate, possesses anticancer properties. Curcumin, a yellow chemical that is a member of the polyphenol superfamily, is what gives turmeric its active flavor. It can cause tumor cells to undergo apoptosis

5.1 Limitations

While the search for natural drugs for the treatment of RA with minimal side effects is a requirement of the moment and phytochemicals show promising results however they come with certain limitations and major setbacks including low oral bioavailability. This problem cannot even be solved by increasing dosage as increasing consumption may result in liver damage along with multiple other side effects including eye irritation, skin irritation, etc., and due to much lower efficacy with short half-life problem remains further unsolved. Phytochemicals are metabolized much rapidly benefits remain minimal. Therefore further studies are required to assess pharmacological interactions with phytochemicals and mechanisms that might be resulting in toxicity of these products.

5.2 Future Scope

Methods such as encapsulation of natural medicines for slow release and reduced side effects, nano-micelles and nanoparticles in combination with herbal medicine for targeted therapeutic approach and use of lipid-core nanocapsules to overcome disadvantages and limitations can be applied. By utilizing these methods certain problems associated with natural drugs including the stability of the drug, the efficacy of the drug, and targeted therapy can be resolved, therefore these combined approaches can revolutionize RA therapy by all-natural drugs.

CHAPTER 6

CONCLUSION

In this study, we have discussed a thorough overview of the usage of phytochemicals as a potential therapy for RA treatment. Many plants found in nature have miraculous properties with abilities to treat a wide variety of disorders and diseases. Plants usually contain a range of substances including sterols, flavonoids, and alkaloids, with anti-inflammatory and anti-oxidant properties. Therefore, the herbal medicine market is in increasing demand due to its fewer to no side effects. In-silico studies have shown promising results for using phytochemicals as inhibitors for JAK2 to regulate the abnormally functioning JAK/STAT pathway in RA.

Using Autodock Vina for molecular docking, phytochemicals' binding affinities and interactions when fitting into the active site were identified. The phytochemical ellagic acid, which is found in fruits and nuts, has the lowest binding affinity and thus the most stable docked complex with JAK2 protein. Additionally, curcumin, andrographolide, and quercetin produced acceptable binding affinities. These phytochemicals' drug-like qualities have also been confirmed in past studies, making them suitable for therapy. Analyzing the structure-activity connections and making the appropriate modifications could improve the inhibitory effect of JAK2 and make them effective anticancer drugs.

References

[1] R. Harrington, S. A. Al Nokhatha, and R. Conway, "JAK Inhibitors in Rheumatoid Arthritis: An Evidence-Based Review on the Emerging Clinical Data," *JIR*, vol. Volume 13, pp. 519–531, Sep. 2020, doi: 10.2147/JIR.S219586.

[2] A. Morinobu, "JAK inhibitors for the treatment of rheumatoid arthritis," *Immunological Medicine*, vol. 43, no. 4, pp. 148–155, Oct. 2020, doi: 10.1080/25785826.2020.1770948.

[3] A.-F. Radu and S. G. Bungau, "Management of Rheumatoid Arthritis: An Overview," *Cells*, vol. 10, no. 11, p. 2857, Oct. 2021, doi: 10.3390/cells10112857.

[4] Y.-J. Lin, M. Anzaghe, and S. Schülke, "Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis," *Cells*, vol. 9, no. 4, p. 880, Apr. 2020, doi: 10.3390/cells9040880.

[5] J. Bullock *et al.*, "Rheumatoid Arthritis: A Brief Overview of the Treatment," *Med Princ Pract*, vol. 27, no. 6, pp. 501–507, 2018, doi: 10.1159/000493390.

[6] L. S. Simon *et al.*, "The Jak/STAT pathway: A focus on pain in rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 51, no. 1, pp. 278–284, Feb. 2021, doi: 10.1016/j.semarthrit.2020.10.008.

[7] C. J. Malemud, "The role of the JAK/STAT signal pathway in rheumatoid arthritis," *Therapeutic Advances in Musculoskeletal*, vol. 10, no. 5–6, pp. 117–127, Jun. 2018, doi: 10.1177/1759720X18776224.

[8] Q. Ding *et al.*, "Signaling pathways in rheumatoid arthritis: implications for targeted therapy," *Sig Transduct Target Ther*, vol. 8, no. 1, p. 68, Feb. 2023, doi: 10.1038/s41392-023-01331-9.

[9] Y. Gandhi *et al.*, "Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review," *Food Chemistry Advances*, vol. 1, p. 100085, Oct. 2022, doi: 10.1016/j.focha.2022.100085.

[10] A. S. Achutha, V. L. Pushpa, and K. B. Manoj, "Comparative molecular docking studies of phytochemicals as Jak2 inhibitors using Autodock and ArgusLab," *Materials Today: Proceedings*, vol. 41, pp. 711–716, 2021, doi: 10.1016/j.matpr.2020.05.661.

[11] R. K. Aravilli, S. L. Vikram, and V. Kohila, "Phytochemicals as potential antidotes for targeting NF-κB in rheumatoid arthritis," *3 Biotech*, vol. 7, no. 4, p. 253, Aug. 2017, doi: 10.1007/s13205-017-0888-1.

[12] G. Kour, S. A. Haq, B. K. Bajaj, P. N. Gupta, and Z. Ahmed, "Phytochemical add-on therapy to DMARDs therapy in rheumatoid arthritis: In vitro and in vivo bases, clinical evidence and future trends," *Pharmacological Research*, vol. 169, p. 105618, Jul. 2021, doi: 10.1016/j.phrs.2021.105618.

[13] G. Kour, R. Choudhary, S. Anjum, A. Bhagat, B. K. Bajaj, and Z. Ahmed, "Phytochemicals targeting JAK/STAT pathway in the treatment of rheumatoid arthritis: Is there a future?," *Biochemical Pharmacology*, vol. 197, p. 114929, Mar. 2022, doi: 10.1016/j.bcp.2022.114929.

[14] L. E. Mendie and S. Hemalatha, "Bioactive Compounds from Nyctanthes arbor tristis Linn as Potential Inhibitors of Janus Kinases (JAKs) Involved in Rheumatoid Arthritis," *Appl Biochem Biotechnol*, vol. 195, no. 1, pp. 314–330, Jan. 2023, doi: 10.1007/s12010-022-04121-1.

[15] S. Nakayamada, S. Kubo, S. Iwata, and Y. Tanaka, “Recent Progress in JAK Inhibitors for the Treatment of Rheumatoid Arthritis,” *BioDrugs*, vol. 30, no. 5, pp. 407–419, Oct. 2016, doi: 10.1007/s40259-016-0190-5.

[16] M. Harigai, “Growing evidence of the safety of JAK inhibitors in patients with rheumatoid arthritis,” *Rheumatology*, vol. 58, no. Supplement_1, pp. i34–i42, Feb. 2019, doi: 10.1093/rheumatology/key287.

[17] J. Angelini *et al.*, “JAK-Inhibitors for the Treatment of Rheumatoid Arthritis: A Focus on the Present and an Outlook on the Future,” *Biomolecules*, vol. 10, no. 7, p. 1002, Jul. 2020, doi: 10.3390/biom10071002.

[18] K. Yamaoka, “Janus kinase inhibitors for rheumatoid arthritis,” *Current Opinion in Chemical Biology*, vol. 32, pp. 29–33, Jun. 2016, doi: 10.1016/j.cbpa.2016.03.006.

[19] L. Â. M. Santiago *et al.*, “Flavonoids, alkaloids and saponins: are these plant-derived compounds an alternative to the treatment of rheumatoid arthritis? A literature review,” *Clin Phytosci*, vol. 7, no. 1, p. 58, Dec. 2021, doi: 10.1186/s40816-021-00291-3.

[20] K. Yuan *et al.*, “Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities,” *The Journal of Nutritional Biochemistry*, vol. 84, p. 108454, Oct. 2020, doi: 10.1016/j.jnutbio.2020.108454.

[21] Z. Li, J. Tan, L. Wang, and Q. Li, “Andrographolide Benefits Rheumatoid Arthritis via Inhibiting MAPK Pathways,” *Inflammation*, vol. 40, no. 5, pp. 1599–1605, Oct. 2017, doi: 10.1007/s10753-017-0600-y.

[22] G. Allam, E. A. Mahdi, A. M. Alzahrani, and A. S. Abuelsaad, “Ellagic acid alleviates adjuvant induced arthritis by modulation of pro- and anti-inflammatory cytokines,” *cejoi*, vol. 4, pp. 339–349, 2016, doi: 10.5114/ceji.2016.65132.

[23] M. G. Alexandrow, L. J. Song, S. Altiok, J. Gray, E. B. Haura, and N. B. Kumar, "Curcumin: a novel Stat3 pathway inhibitor for chemoprevention of lung cancer," *European Journal of Cancer Prevention*, vol. 21, no. 5, pp. 407–412, Sep. 2012, doi: 10.1097/CEJ.0b013e32834ef194.

[24] J. J. Bright, "CURCUMIN AND AUTOIMMUNE DISEASE," in *The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease*, vol. 595, B. B. Aggarwal, Y.-J. Surh, and S. Shishodia, Eds., in ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, vol. 595. , Boston, MA: Springer US, 2007, pp. 425–451. doi: 10.1007/978-0-387-46401-5_19.

[25] D. Khanna *et al.*, "Natural products as a gold mine for arthritis treatment," *Current Opinion in Pharmacology*, vol. 7, no. 3, pp. 344–351, Jun. 2007, doi: 10.1016/j.coph.2007.03.002.

[26] Computational Biology Laboratory, Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India, Biotech Hub, Centre for the Environment, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India, A. Kumar, and U. Bora, "Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids," *Bioinformation*, vol. 8, no. 20, pp. 988–993, Oct. 2012, doi: 10.6026/97320630008988.

[27] J. Kirchmair *et al.*, "The Protein Data Bank (PDB), Its Related Services and Software Tools as Key Components for In Silico Guided Drug Discovery," *J. Med. Chem.*, vol. 51, no. 22, pp. 7021–7040, Nov. 2008, doi: 10.1021/jm8005977.

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