Bachelor of Pharmacy

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2024

Computational Investigation of Thymus linearis Phytoconstituents for the Treatment of Rheumatoid Arthritis: A Promising Approach towards Herbal Therapy

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BACHELOR OF PHARMACY

UNDER THE GUIDANCE OF

Dr. BHUMIKA D. PATEL

INSTITUTE OF PHARMACY
NIRMA UNIVERSITY

MAY 2024

Computational Investigation of Thymus linearis Phytoconstituents for the Treatment of Rheumatoid Arthritis: A Promising Approach towards Herbal Therapy

A Thesis submitted to the Institute of Pharmacy, Nirma University, in partial fulfilment of the requirements for the Degree of

BACHELOR OF PHARMACY

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Semester VIII

(PROJECT WORK BP812PW)

UNDER THE GUIDANCE OF

DR. BHUMIKA D. PATEL
INSTITUE OF PHARMACY
NIRMA UNIVERSITY

MAY 2024

DECLARATION

We, **ARYAN SORATHIA (20BPH013), BHAVYAKUMAR NAKUM (20BPH016), MADHURIMA MANDAL (20BPH059), SHRIYA MENPARA (20BPH093)**, hereby declare that B. Pharm project work (BP812PW) entitled **"COMPUTATIONAL INVESTIGATION OF THYMUS LINEARIS PHYTOCONSTITUENTS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS: A PROMISING APPROACH TOWARDS HERBAL THERAPY"** being submitted to Institute of Pharmacy, Nirma University for the award of degree of B. Pharm was carried by us/me under the supervision of **Dr. Bhumika D. Patel**, Institute of Pharmacy, Nirma University. The content of this project work, in full or in parts, has not been submitted to any other University for the award of any degree. We also declare that all the information collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.

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CERTIFICATE FOR SIMILARITY OF WORK

This is to undertake that the B. Pharm Project work (BP812PW) entitled **"COMPUTATIONAL INVESTIGATION OF THYMUS LINEARIS PHYTOCONSTITUENTS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS: A PROMISING APPROACH TOWARDS HERBAL THERAPY"**. Submitted by **ARYAN SORATHIA (20BPH013), BHAVYAKUMAR NAKUM (20BPH016), MADHURIMA MANDAL (20BPH059), SHRIYA MENPARA (20BPH093)**, B.Pharm. Semester VIII is a Bonafide research work carried out by us at the Institute of Pharmacy, Nirma University under the guidance of Dr. Bhumika D. Patel. We are aware about the rules and regulations of the Plagiarism policy of Nirma University, Ahmedabad.

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Date: 14/ 05 /2024

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B.D.Patel

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Date: 14/ 05 /2024

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CERTIFICATE

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ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to **Dr. Bhumika D. Patel**, our thesis guide, for her invaluable guidance, patience, and profound expertise, which were crucial to the development and completion of our thesis. Dr. Bhumika D. Patel 's thorough approach and meticulous attention to detail have significantly enhanced our research skills and understanding of the subject matter. Her encouragement and constructive criticism have been indispensable throughout this challenging yet rewarding journey.

We take this opportunity to express our profound gratitude to **Dr. Karsanbhai K. Patel**, Founder-President, Nirma University for his support by providing the world class facility to carry out this research work.

We are also immensely grateful to Director **Dr. Gopal Natesan**, whose visionary leadership and scholarly insights have profoundly influenced our academic experience. His strategic directions and motivational talks have played a pivotal role in shaping our approach to research and our professional growth.

And special thanks are also due to **Dr. Hardik Bhatt**, the Head of our Department, for his academic and administrative support. Dr. Bhatt's leadership and commitment to excellence have greatly enriched our educational experience and provided us with numerous opportunities for learning and growth.

We extend our heartful gratitude to **Dr. Udit Chaube**, the Assistant Professor, for his academic and administrative support. Dr. Chaube' s leadership and commitment to excellence have greatly enriched our educational experience and provided us with numerous opportunities for learning and growth.

We extend our heartfelt gratitude to all the participants who graciously took the time from their busy schedules to respond to our questionnaire.
We are most thankful to our parents, without whom it would have been next to impossible to complete this thesis. Their help, kind support, understanding, encouragement and constant motivation have always kept us going in our life.

Lastly, we thank the Almighty, who has given us this opportunity to extend our gratitude to all those people who have helped us and guided us throughout our research work and life.

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Abbreviations

1. Tak- Transforming growth factor beta activated kinase-1
2. RA- Rheumatoid arthritis
3. Jak- The Janus kinase
4. ADMET- Absorption, Distribution, Metabolism, Excretion and Toxicity.
5. COX- Cyclooxygenase
6. IL6- Interleukin-6
7. ACPA- anti-citrullinated protein/peptide antibody
8. RF- rheumatoid factor
9. NSCLC- Non-Small Cell Lung Cancer
10. LC- Lung cancer
11. MTX- Methotrexate
12. DMARDs- Disease-modifying antirheumatic drugs
13. IBD- Inflammatory bowel disease
14. TGF beta- Transforming growth factor-β
15. TNF alpha- Tumor Necrosis Factor alpha
16. IFN gamma- Interferon-gamma
17. MIC- Minimum inhibitory concentrations
18. MBC- Minimum bactericidal concentration
19. CRP- C-reactive protein
20. RMS- Root mean square
21. DH- Docking hits
22. RMSF- Root mean square fluctuation

Abstract

The current study investigated several popular artificial algorithms for efficient hit selection, which might be applied to the identification of TAK 1 and JAK inhibitors for the treatment of rheumatoid arthritis. To accomplish the intended objectives Molecular docking experiments, database retrieval, *de novo* drug design, knowledge-based drug design, and molecular dynamics simulations were carried out. Targeting particular inhibitors, such as TAK 1, IL-16, and JAK, is one of the main components of the current tactics for treating inflammation. Our work has significant therapeutic applications as it focuses on exploiting *Thymus linearis* to harness inflammatory activity. Among the six constituents found in the plant, thymol was identified as the primary constituent with the highest concentration and had appropriate ADMET data. Subsequently, two protein candidates were docked with it: 5V5N (TAK1) and 7TEU (JAK), which were recognized as viable ones based on their docking score. Following that, thymol demonstrated a robust binding interaction with TAK1 as well as with JAK which proved its efficacy against the TAK1 and JAK. The outcome of molecular docking and dynamics simulations has confirmed that the Thymol-JAK complex exhibits greater stability compared to the Thymol-TAK complex. However, to enhance the binding affinity towards both proteins further, a suitable hit compound was designed from Thymol using the *de novo* drug design technique. Both the docking and simulation studies demonstrated that the hit (DH) developed from the Thymol exhibited steady and efficient binding energies, indicating that it might serve as a useful treatment against rheumatoid arthritis.

Keywords: Rheumatoid arthritis, TAK1, JAK, *Thymus linearis*, *de novo* drug design, DeepFrag, GOLD

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Chapter 1 Introduction

RA, also referred to as rheumatoid arthritis, is an autoimmune disease that causes inflammation. It develops when your immune system mistakenly attacks healthy cells, causing excruciating swelling in the body's afflicted regions. Usually, the illness begins with the little, peripheral joints of the hands or feet and leads more to proximal joints, such as the knees, shoulders, hips, wrists ankles, and elbows. Untreated RA causes loss of bone & cartilage resulting in deformities and incapacity. Additional indications may encompass symptoms such as exhaustion, debilitation, and elevated body temperature. Pathogenesis of RA is shown in the Fig. 1.1

Since the year 2019, much evidence indicates that rheumatoid arthritis has caused serious issues by infecting 18 million people worldwide. In 2020, an estimated 17·6 million (95% uncertainty interval 15·8–20·3) people had rheumatoid arthritis worldwide. The age- standardized global prevalence rate was 208·8 cases (186·8–241·1) per 100 000 population, representing a 14·1% (12·7–15·4) increase since 1990. As per the data, individual diagnoses of rheumatoid arthritis are nearly 70% female, and 55% of cases occurred in people aged 55 years or older. Thirteen millions of these individuals, are diagnosed with illnesses that have varying degrees of severity; hence, to overcome these rehabilitation interventions, which are advantageous and have potential, opportunities for rehabilitation interventions to be advantageous (Cieza et al. 2006; http://www.who.int/emergencies/disease-outbreak-news/iten/2024-DON512 ; Vos et al. 1204)

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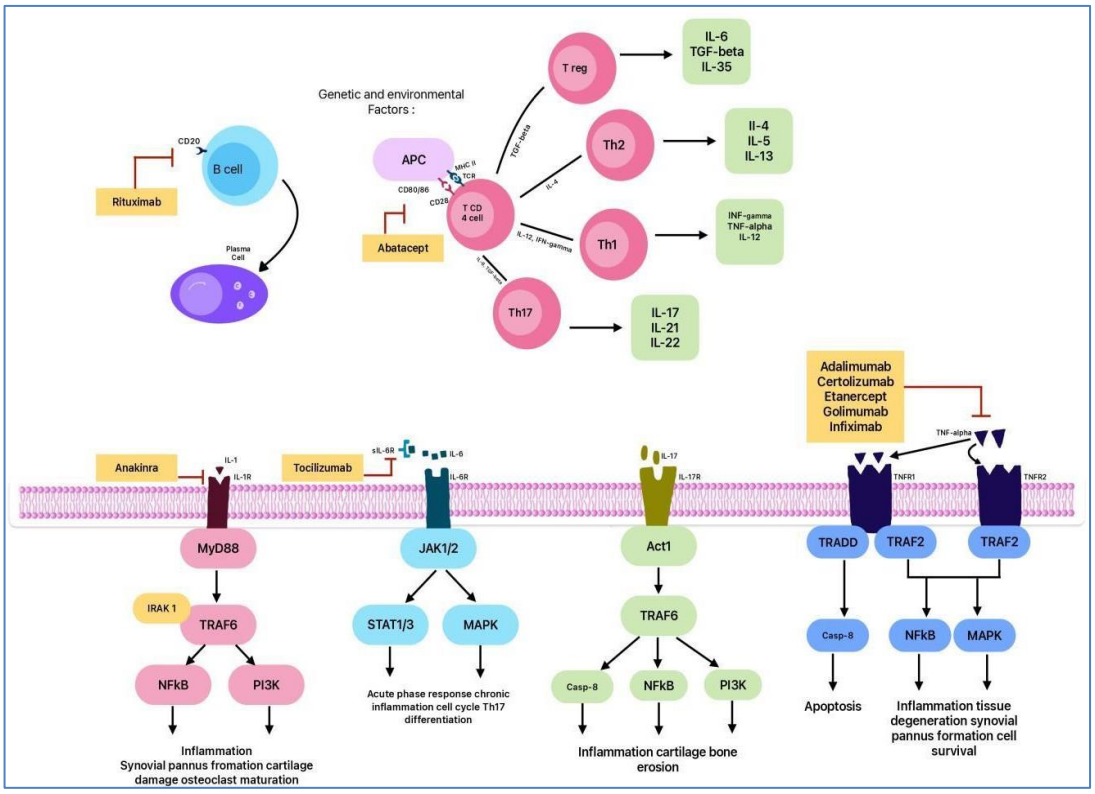


Fig. 1.1 *Pathogenesis of Rheumatoid arthritis and therapeutic targets. Here, APCs stands for antigen-presenting cells, TAK 1 AND JAK-α as tumor necrosis factor α, IL as interleukin, Th as T-helper, JAK Janus Kinase, STAT signal transducers and activator of transcription, TRAF tumor necrosis factor receptor-associated factor, TRADD tumor necrosis factor receptor- associated death domain, PI3K phosphoinositide 3-kinase, MMPs matrix metalloproteinases, GM-CSF granulocyte–macrophage colony-stimulating factor, RANKL receptor activator of nuclear factor kappa-B ligand. Effective therapeutic targets are shown here are: antibody anti IL-6 receptor (tocilizumab), an IL-1 antagonist and a small molecule that inhibits JAK (tofacitinib) signaling, inhibition of T cell co-simulation (abatacept), and depletion of B cells with anti-CD20 antibody (rituximab) (Chaube et al.)*

Rheumatoid arthritis is diagnosed based on a person's symptoms, a physical examination, and the outcomes of blood, scan, and x-ray tests. Because there is no test that can conclusively establish a person have it, diagnosis might be challenging. Additionally, a number of illnesses share similar symptoms. In addition, doctor will examine a person physically. They will assess the range of motion in joints and search for any swelling. It's crucial to report to doctor all of the symptoms, even if they don't appear connected, as rheumatoid arthritis can affect multiple body regions at

once. In order to diagnose the illness rheumatologists will arrange blood tests, although there is no single blood test that can confirm a person have rheumatoid arthritis but some show possible signs of the condition. Those main tests are mentioned below:

1. Erythrocyte sedimentation rate (ESR)
2. C-reactive protein (CRP)
3. Full blood count
4. Rheumatoid factor and anti-CCP antibodies

Also, there are some scans used to check for joint inflammation and damage. These may include x-rays (they will show any changes in joints), ultrasound scans (shows picture of the joints using high-frequency sound waves), magnetic resonance imaging scans (shows pictures of joints produced using strong magnetic fields and radio waves).

There is plethora of treatments available for rheumatoid arthritis. Mainly it can be treated in three ways:

1. Drugs

There are 4 groups of drugs that are used to treat rheumatoid arthritis which includes painkillers, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti- rheumatic drugs (DMARDs) and steroids (also known as corticosteroids).

2. Physical therapies
3. Surgery

Interestingly, a wide range of medications have been found to date, that function by inhibiting various proteins which include Interleukin-6 (IL-6), T-cell blocking agent, transforming growth factor-β-activated kinase 1(TAK1) inhibitor, Janus kinase (JAK) inhibitor, Cyclooxygenase (COX) inhibitor.

The pro-inflammatory cytokine IL-6 contributes to many different facets of the RA pathogenesis. It boosts the synthesis of acute-phase reactants such C-reactive protein (CRP) and promotes the transformation of B cells into plasma cells that release antibodies. Tumor necrosis factor-alpha (TNF-α) and IL-1β-activated signaling pathways, are partly mediated by TAK1. Through the activation of downstream transcription factors like AP-1 (Activator Protein-1) and NF-κB (Nuclear Factor-kappa B), it

regulates the expression of genes that are associated with inflammation. TAK1 is also essential for the survival and development of immune cells implicated in the pathophysiology of RA. Janus kinases are intracellular enzymes that transmit signals from cytokine receptors to the nucleus, regulating gene expression and immune cell function. JAK is responsible for chronic inflammation and joint damage. COX enzymes responsible for the synthesis of prostaglandins which plays roles in inflammation, pain, and fever.

Many adverse effects have been reported with the existing therapeutic options, including: Firstly, there is a substantial body of evidence indicating that the already accessible medications are exhibiting unfavorable effects, notably pulmonary toxicity, which has been observed to occur more frequently in individuals with rheumatoid arthritis (RA) who are undergoing treatment with methotrexate (MTX) (Helliwell and Taylor 472).

Further, there exists a well-documented and quantifiable correlation between the utilization of TAK1 and JAK inhibitor monotherapies by patients with rheumatoid arthritis and the occurrence of psoriasis as an adverse event. All TAK1 and JAK inhibitor medications; Certolizumab pegol, Adalimumab, Golimumab, Infliximab, Etanercept, Tofacitinib, Baricitinib and Upadacitinib (Fraenkel et al 924; Harrington et al. 519) that have received approval for the treatment of rheumatoid arthritis (RA), as well as three out of the four non- TAK1 inhibitor drugs that were examined, demonstrated a statistically significant correlation with psoriasis when compared to methotrexate. Among the TAK1 inhibitors that were examined, certolizumab pegol had the most frequently reported occurrence of psoriasis (Joulfayan et al.). Finally, RA can be treated long-term with hydro chloroquine, also known as Plaquenil, an antimalarial drug. This drug lessens the amount of proinflammatory cytokines that monocytes release.

Issues with the skin, central nervous system, and gastrointestinal tract are typical adverse effects. When this medication is used in large amounts, it can specifically harm the eyes. Patients using this drug should see an ophthalmologist on a regular basis(Bullock et al.501;Da Silva et al.766). JAK inhibitors have the potential to reduce immunological response, which could affect the host's ability to fight off infections. Nasopharyngitis and upper respiratory tract infections (URIs) are the two infections that are most commonly reported shown in Table 1.1.

Table 1.1 Few available drugs for the Rheumatoid Arthritis and their side effects

Sr. No.	**Drug**	**Side-effects**
1.	Methotrexate	Unfavorable effects like pulmonary toxicity
2.	Certolizumab	It caused psoriasis, a chronic illness where skin cells proliferate too quickly due to an overactive immune system.
3.	Hydroxychloroquine	Complications with the skin, central nervous system, and gastrointestinal tract. At higher concentration, it can specifically harm eyes.
4.	JAK inhibitors	Nasopharyngitis and upper respiratory tract infections

All available data suggest that, there is an enormous demand for new drugs with a higher therapeutic index and fewer side effects. An efficient medicinal remedy to counteract the negative effects of currently accessible medication that operated as a potential JAK and TAK1 inhibitor was identified as a naturally occurring plant. *Thymus linearis* belonging to family Lamiaceae is known as Thyme. The plant has been proved to have antibacterial (Gilani et

al.987) antiviral (Hafidh et al.58) and anti-cancer activity (Hussain et al. 249) which contains: Thymol (52.28–66.65%), *p*-cymene (1.81–21.60%) and γ-terpinene (1.94–12.48%). Other constituents identified in significant amounts were carvacrol, *p*-cymen-8-ol, borneol, terpinen-4-ol and thymol methyl ether. The presence of high phenol and essential oil contents in this species make it a suitable substitute for common thyme oil (Verma et al 1890). It has been documented that *Thymus linearis* is used in folklore (Qadir et al.591) to treat fever, inflammation, and pain. Plant extracts haven't, however, undergone pharmacological evaluation for the aforementioned activities. Thus, the goal of this study was to assess *Thymus Linearis*'s analgesic, anti-inflammatory, and antipyretic properties in order to verify its traditional claim (Hamzah et al).

In our research, Thymol demonstrated a strong binding affinity to JAK compared to TAK1, as revealed by molecular docking-assisted molecular dynamics analysis. Building on this finding, Thymol underwent optimization using de novo drug design, facilitated by DeepFrag software. The resulting designed compound exhibited encouraging efficacy against JAK and holds potential for further investigation as a treatment option for rheumatoid arthritis

Chapter 2 Literature Review

Thymus linearis plant is taken in order to check its activity against rheumatoid arthritis. So, in order to test the efficacy of thymus its 6 constituents are taken which are Thymol, p-cymene, gamma terpinene, carvacrol, linalool, beta caryophyllene. Receptors like TAK-1, JAK, COX and IL-6 are found to be responsible for the Rheumatoid Arthritis.

Rheumatoid Arthritis:

Scherer et al. (102400) cover the complexities of rheumatoid arthritis (RA), focusing on its heterogeneity and the early involvement of immune responses prior to clinical symptoms onset. The division of RA into ACPA-positive and -negative subgroups based on genetic and autoantibody data is highlighted. ACPA, in example, is exclusive to RA, whereas RF can be observed in other diseases. Genetic factors such as shared epitope alleles predispose to ACPA- positive RA, and smoking is a major environmental risk factor.

Immunological alterations, including both adaptive and innate immune responses, play critical roles in synovitis, and new study suggests possible treatment targets. While current treatments primarily target immunosuppression, there is significant interest in microbiome-related factors and their impact on RA. The abstract proposes a relationship between microbial triggers, innate immune activation, and adaptive immunity in RA pathogenesis.

The abstract closes by appreciating progress in understanding RA while emphasizing the need for additional research into its origins and potential microbiological triggers. It also pays honor to Josef Smolen, a well-known pioneer in RA research and treatment, recognizing his efforts to understanding RA pathophysiology and creating therapeutic approaches. His research on targeted therapy and management guidelines has greatly benefited RA sufferers.

2.1 Target inhibitor of Thymus linearis

2.1.1 Transforming growth factor beta activated kinases-1 (TAK1)

Zhu et al. (453) Investigate the role of Transforming Growth Factor β Activated Kinase 1 (TAK1) in angiogenesis, specifically in pathological situations such tumor angiogenesis and retinal neovascularization. It focuses on TAK1 as a crucial mediator in a variety of physiological activities, including immunological responses, cell survival, and death, as well as its activation in response to stimuli such as pro-inflammatory cytokines, hypoxia, and oxidative stress.

The review summarizes the processes by which TAK1 regulates pathological angiogenesis and examines prospective TAK1-targeted therapies. It stresses the significance of knowing the interactions between TAK1 and other signaling pathways involved in angiogenesis-related diseases.

Furthermore, the abstract states that pharmacological inhibitors and genetic techniques targeting TAK1 have showed promise in a variety of malignancies, improving chemotherapeutic efficiency by modulating inflammatory and angiogenic processes. However, it recognizes the need for additional research to completely understand TAK1's role in angiogenesis and related illnesses, as well as its interaction with downstream signaling pathways under various situations.

Finally, TAK1 emerges as a possible therapeutic target for pathological angiogenesis, providing an alternative or complementary approach to existing treatments. However, more extensive research is required to completely understand its mechanics and possible clinical applications.

2.1.2 Cyclooxygenase (COX)

Liu et al. determines that lung cancer (LC), more specifically non-small cell lung cancer (NSCLC), is the largest cause of cancer-related death worldwide. The stress-induced enzyme COX-2 plays an important role in LC advancement by boosting prostaglandin E2 synthesis, which facilitates tumors incidence and development. Early-stage NSCLC patients with COX- 2 overexpression may benefit from targeted therapy, but combining COX-2 inhibitors with other modalities improves therapeutic efficacy while minimizing deleterious effects on healthy tissues, potentially increasing overall patient survival rates after treatment. Targeting COX-2 inhibition in NSCLC is a viable therapeutic approach, with possible COX-2 regulators functioning as useful LC indicators.

Combining COX-2 inhibitors with traditional therapies such as chemotherapy and radiotherapy offers an approach for overcoming drug resistance in LC, paving the way for novel and successful therapeutic options. To summarize, COX-2 inhibition is a potential method to NSCLC treatment, offering opportunities for improving prognosis and treating this aggressive malignancy. This review emphasizes the importance of COX-2 as a therapeutic target and suggests new techniques for NSCLC therapy.

2.1.3 Interleukin-6 (IL6)

Grebenciucova and VanHaerent shows multifunctional cytokine, interleukin 6 (IL-6) has a variety of roles in both human health and disease. IL-6 is produced by a variety of cell types, such as fibroblasts, endothelial cells, and immune cells. It is essential for tissue homeostasis, inflammation, and immunological control. The complex interactions between IL-6 and several physiological systems are examined in this literature review, along with the consequences of this interaction in a variety of clinical situations. Owing to its pivotal function in immune regulation and inflammation, IL-6 has surfaced as a potential therapeutic target for an array of ailments. Patients with rheumatoid arthritis have

found remission from inflammatory and autoimmune diseases with the use of biologic medicines that target IL-6 or its receptor.

2.1.4 Janus kinase (JAK)

Taylor et al. (17) explains about clinical efficacy and the additional treatments like methotrexate (MTX) are effective for some but not all patients, leading to the exploration of Janus kinase (JAK) inhibitors like tofacitinib and Baricitinib. Clinical trials have shown significant improvements in RA symptoms and functionality with these drugs, whether used alone or with MTX, demonstrating their disease-modifying potential. Organizations like the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) have guidelines that suggest beginning with MTX and moving to JAK inhibitors if responders are insufficient or if six months pass with no discernible improvement. Tofacitinib, the first oral JAK inhibitor approved for RA, and Baricitinib have both shown efficacy in reducing disease activity, improving physical function, and slowing joint damage progression.

These drugs offer rapid relief, oral administration, and targeted inflammatory pathway inhibition, making them valuable options for patients with moderate to severe RA who have not responded adequately to traditional therapies or biologic DMARDs (bDMARDs). Continued research will further refine our understanding of their long-term safety and effectiveness, solidifying the role of JAK inhibitors in the evolving landscape of RA treatment.

2.2 Constituents of thymus linearis

2.2.1 Thymol

Y. Liu et al. (1947) offers a complete analysis of thymol's potential benefits in the treatment of inflammatory bowel disease (IBD). Thymol, a natural chemical found in thyme, has received attention for its several biological roles, including its potential as an adjuvant therapy for IBD.

The specific etiology of IBD is unknown, but environmental factors, genetics, stress, food, and immune system dysfunction all contribute to its

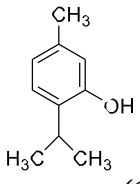
development and progression. Thymol has showed potential in reducing some of the variables related with IBD.

The review emphasizes the impact of nutrition in IBD. Dietary variables can alter gut microbiota composition, mucosal defenses, and oxidative stress levels, all of which are linked to IBD etiology. Thymol's characteristics appear to benefit gut health by increasing gut integrity, lowering oxidative stress, regulating immunological responses, and preserving a healthy intestinal microenvironment.

Furthermore, the review suggests that thymol may have advantages over traditional IBD medications, which are frequently associated with serious adverse effects. Herbal extracts such as thymol, with its antioxidant effects and minimal toxicity, offer a viable option for supplemental therapy in IBD treatment.

Despite the possible benefits indicated in animal studies, the review concludes that more research, particularly clinical trials, is required to test thymol's efficacy and determine ideal dosages for human ingestion. Furthermore, knowing the precise mechanisms by which thymol exerts its therapeutic benefits on mucosal immunity and oxidative stress in the intestinal tract is a topic of ongoing research.

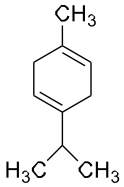
In conclusion, while additional research is needed, thymol emerges as a promising supplementary therapy for IBD due to its gut health benefits and natural, non-toxic profile.



(1)

2.2.2 Gamma-terpinene

Nooshadokht et al. (109957) covers that leishmaniasis is an important global health concern, with fewer therapeutic options. This study looks into the biological effects of gamma-terpinene (GT) on Leishmania major and its potential as an antileishmanial agent. GT's significant effectiveness against L. major stages suggests an immunomodulatory role, upregulating ions and JAK-1 while downregulating IL-10 and TGF-β. In addition, GT has antioxidative characteristics and induces macrophages to remove the organism. Additional in vivo and clinical investigations are needed to determine GT's efficacy in future treatment plans. The findings underscore GT's intriguing significance in leishmaniasis treatment, providing insights into its mode of action and possible benefits for therapy. However, much research must be conducted to determine its safety profile, dose regimen, and long-term efficacy. GT emerges as a promising candidate for antileishmanial therapy, but its clinical utility awaits validation through rigorous experimentation and clinical trials.

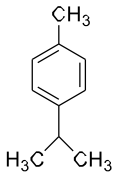


(2)

2.2.3 P-cymene

(3) Sani et al. (1) looks at p-cymene's antidiabetic effects in male Wistar rats with streptozotocin-induced diabetes. P-cymene, which has been recognized for its antioxidant and anti-inflammatory characteristics, was tested against metformin. The results established p- cymene's potential to enhance glucose and lipid profiles, lower liver enzyme levels, and reduce the effects of oxidative stress. Furthermore, p-cymene regulated the Akt/mTOR signaling pathway, reducing hepatic and pancreatic damage. These findings point to its potential as a hypoglycemic, hypolipidemic, and antioxidant medication, with implications for diabetes therapy both alone and in combination with metformin. The study emphasizes the role of hyperglycemia, liver injury, oxidative stress, and Akt/mTOR pathway inhibition in diabetes progression. P-cymene treatment considerably reduced these variables, demonstrating its

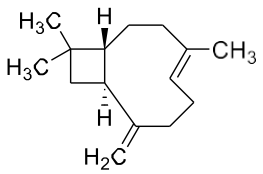
prospective anti-diabetic properties. However, additional research is needed to identify the appropriate dosage, treatment duration, and potential interactions with other supplements. Despite these drawbacks, p-cymene has emerged as a comparable supplementary treatment to metformin for diabetic patients. Its mechanisms of action require further investigation to fully comprehend its antidiabetic characteristics and therapeutic potential.



(3)

2.2.4 Beta Caryophyllene

Gushiken et al. (1) examine the ability of β-caryophyllene to heal wounds using a rat skin wound excision model, emphasizing the substance's anti-inflammatory, re-epithelialization, and antioxidant properties. β-caryophyllene has significant anti-inflammatory properties at the site of wounds. Biochemical tests revealed decreased levels of pro-inflammatory cytokines, including TNF-α, IFN-γ, IL-1β, and IL-6. By inhibiting inflammation, β-caryophyllene could provide an environment that is better for wound healing, minimizing tissue damage and quickening the healing process. The research conducted by Gushiken et al. highlights the potential of β-caryophyllene as a versatile drug that can facilitate wound healing. Its capacity to improve re-epithelialization and tissue remodeling speeds up wound closure, while its antioxidant and anti-inflammatory qualities reduce oxidative stress and inflammation.



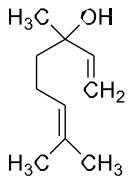
(4)

2.2.5 Linalool

According to Bakkali et al. (1183), the review highlights the importance of linalool in a number of industries while examining its biological activity, medicinal potential, and commercial applications. First, Bakkali et al. clarify the chemical makeup and origins of linalool. Plant species including citrus, lavender, and menthe are frequently used to extract linalool, an alcohol derived from cyclic terpenes that has a flowery scent The review discusses the many biological activities of linalool, such as its analgesic, antioxidant, and antibacterial properties in addition to its anti-inflammatory and anti-inflammatory properties.

Furthermore, linalool has been shown to have neuroprotective properties against oxidative stress and neurodegenerative illnesses, indicating prospective uses in neurotherapeutics.

Linalool is an essential component of perfumery, cosmetics, and aromatherapy products due to its pleasant aroma and physiologically active qualities. Additional investigation into the mechanisms of action and therapeutic uses of linalool is promising in terms of the creation of novel products and treatments that improve human health and welfare.



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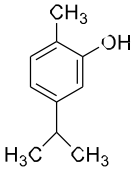
2.2.6 Carvacrol

According to Cacciatore et al. (0120937) antimicrobial resistance poses a serious threat since it reduces the efficacy of conventional antibiotics against resistant bacterial and fungal species. Strong antimicrobial properties can be discovered in carvacrol, a naturally occurring substance present in essential oils such as thyme. However, issues like limited solubility and stability make it difficult to apply. In order to improve its antimicrobial activity, researchers created 10 carvacrol codrugs in order to address this. Out of all of them, Ac-Cys (Allyl)-CAR (codrug 4) shown a noteworthy improvement in antibacterial activity against a range of bacterial strains, with low MIC and MBC values, as well as good antifungal activity against Candida albicans. The results of toxicity tests suggested that codrug 4 was not very harmful, and when it came to the mature biofilms that Escherichia coli created, it showed signs of being effective against infections linked to biofilms.

According to mechanistic research, codrug 4 may damage microbial cell membranes and cause cell death. Subsequent paths involve assessing antibacterial activity in vivo and ester bond hydrolysis to liberate free carvacrol and Ac- Cys (Allyl)-OH. Good stability is suggested by pharmacokinetic data, which also emphasize the potential of carvacrol codrugs as novel antimicrobial agents against pathogens resistant to antibiotics. These findings underscore the significance of natural compounds and creative approaches in countering antimicrobial resistance and creating novel therapies for infectious diseases.



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CHAPTER 3 Aim & Objective

Aim

The aim of this research is to use computational modeling to investigate Thymus Linearis phytoconstituents for the treatment of Rheumatoid Arthritis

Objective

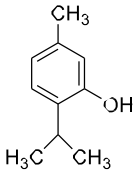
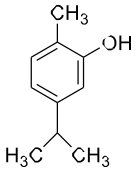
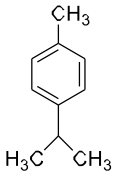
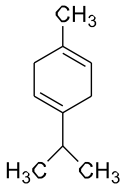
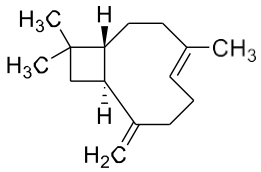
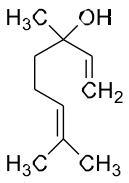
1. Investigate artificial algorithms for effective hit selection against TAK 1 and JAK inhibitors.
2. Use molecular docking experiments, database retrieval, and molecular dynamics simulations to find potential inhibitors.
3. To treat RA, target particular inflammatory pathways involving TAK 1, IL-16, and JAK.
4. Look into the medicinal potential of Thymus linearis components, namely thymol, in moderating inflammatory activity.
5. Dock thymol with the TAK1 and JAK proteins to assess binding interactions and effectiveness.
6. Create hit compounds from thymol utilizing de novo drug design strategies to improve binding affinity to TAK1 and JAK.
7. Conduct docking and simulation tests to determine the stability and efficiency of the planned hit.
8. Determine whether the designed hit compound is a realistic treatment choice for rheumatoid arthritis.

CHAPTER 4 Methodology

4.1 Material and Methods

Thyme is a little, aromatic shrublet that grows to a height of 15 to 30 cm. It has numerous tiny, oblong leaves and delicate whorls of pink-purple flowers that grow in rather thick clusters and often crowd over the leaves. The leaves and blossoms of the Thymus species are used extensively in traditional medicine as herbal teas, tonics, antiseptics, carminatives, and remedies for colds(Verma et al. 557; Figueiredo et al. 3120). The medicinal, cosmetic, fragrance, and flavor industries all make significant use of thyme oils and extracts. It is used in the taste industry to flavor mouthwashes, chocolates, toothpastes, and cough medications. Additionally, it is used to preserve a variety of culinary items(Surburg and Panten ; Mahboubi et al. 395). The most important components in the essential oils of this genus are thymol and carvacrol, followed by beta-caryophyllene, linalool, p-cymene, γ- terpinene, borneol, terpinen-4-ol, and 1,8-cineole (Golbahari and Meysam 890), shown in Table 2.1 along with their chemical structures. Even with improvements in RA management and treatment, the rise of adverse effects has made eradication attempts extremely difficult. The creation of efficacious herbal medicines is a critical area that requires additional research in drug development against RA (Golbahari and Froushani 117037; Kalbak 185; Murugesan et al. 5038; Milne 2053). The investigation of novel medications and treatment plans is necessary to address this growing problem because of the few and frequently ineffective therapeutic options available for side effects. Therefore, this article focuses on the optimal utilization of the *Thymus linearis* for the creation of treatment options against RA.

Table 2.1 Chemical structure of each constituent present in the Thymus linearis

Sr.No.	Name	Structure
1.	Thymol	
2.	Carvacrol	
3.	p-cymene	
4.	Gamma-terpinene	
5.	Beta-caryophyllene	
6.	Linalool	

4.1.1 Ligand preparation

Preparing ligands is a crucial step in pre docking to ensure precise and dependable molecular docking outcomes. The aim is to improve the structure of ligands for optimal interaction with target proteins, a process involving several essential steps. Ligand preparation encompasses various methods and procedures aimed at enhancing energy landscapes and aligning with docking algorithms. This process is key to advancing drug development by enabling researchers to study ligand-target interactions and lay the foundation for accurate and informative molecular docking study.

The first crucial aspect of ligand preparation is hydrogen assignment and bond order determination. Many ligands sourced from databases like PubChem lack explicit information about hydrogen atoms and the precise arrangement of bonds. This deficiency can lead to inaccuracies during docking simulations, as hydrogen bonding and bond angles play pivotal roles in molecular recognition and binding specificity. Ligand preparation addresses this issue by systematically assigning hydrogen atoms and verifying bond orders, thereby creating a more realistic representation of the ligand's chemical structure.

Additionally, ligand preparation involves energy minimization, a computational technique that optimizes the ligand's conformation and geometry. By utilizing software such as Chemdraw 3D, researchers can calculate the energies associated with different molecular conformations and iteratively refine the structure to achieve a local energy minimum. Energy minimization techniques like MM2 or MMFF94 allow for the adjustment of bond lengths, angles, and torsional angles, effectively reducing steric clashes and strain energy within the ligand molecule. (Jones et al. 727) This process not only enhances the ligand's stability but also improves its compatibility with the target protein, increasing the likelihood of accurate docking predictions.

Moreover, Chemdraw 3D's capabilities extend beyond energy calculations and minimization. The software offers a suite of tools for sketching diverse molecular shapes and templates for biological structures. This functionality is invaluable in visualizing ligand-protein interactions and evaluating potential binding modes. Researchers can explore various molecular conformations and select the most favorable ones for docking simulations, thereby enhancing the efficiency and accuracy of the virtual screening process.

During energy minimization, maintaining a low root mean square (RMS) gradient, such as the specified 0.0100, is essential for ensuring convergence to a stable energy minimum. This meticulous control over optimization parameters contributes to the reliability of ligand preparation and subsequent docking experiments.

In preparation for molecular docking investigations, the finalized ligand structures are transformed into the SDF format. This standardized format preserves essential molecular information, including coordinates, bond connectivity, and atom types, facilitating seamless integration into docking software platforms and ensuring consistent data interpretation across simulations.

4.1.2 *De Novo* Drug Design Enhanced by DeepFrag

Fragment-based drug design is a subset of de novo drug design, a computational approach aimed at creating novel drug candidates from scratch without relying on existing compounds De novo drug design comprises multiple approaches, such as structure-based, ligand-based, and fragment-based design. Small molecular fragments are found and assembled to create larger, more complex compounds with better pharmacological properties in fragment-based drug creation.

De novo drug design leverages computational tools like DeepFrag to identify and optimize lead compounds for improved pharmacological properties. Molecular exploration and optimization through iteration is a strategy that helps create new and effective medication candidates. The DeepFrag software, utilized in this study, specializes in fragment-based drug design to optimize lead compounds further. To begin, the structures of both the lead compound (thymol) and the target protein are loaded into the program. Specific substitution sites, denoted as sites A, B, C, and D, are defined based on structural considerations or known binding interactions, as indicated in Table 2.2, 2.3, 2.4, 2.5 respectively.

Rank	SMILES	Structure	Score
1	*NCC		0.574
2	*NC(C)=O		0.567
3	*NC		0.565
4	*NCCO		0.559
5	*N(C)CC		0.555
6	*NC=O		0.550
7	*N(C)C		0.549
8	*NC(=O)CO		0.538

Table 2.2 Possible Substitutions at position A identified through the DeepFrag software

Table 2.3 *Possible Substitutions at position B identified through the DeepFrag software*

Rank	SMILES	Structure	Score
1	*C		1.000
2	*CC		0.582
3	*CO		0.579
4	*CF		0.578
5	*C#N		0.578
6	*CN		0.578
7	*C=O		0.578
8	*C=C		0.578

Table 2.4 *Possible Substitutions at position C identified through the DeepFrag software*

Rank	SMILES	Structure	Score
1	*O		0.818
2	*OC		0.614
3	*OCC		0.511
4	*ON		0.479
5	*OO		0.476
6	*OCCN		0.471
7	*OCCO		0.444
8	*OCCC		0.440

Table 2.5 *Possible Substitutions at position D identified through the Deep Frag software*

Table 2.5 Possible Substitutions at position D identified through the Deep Frag software

Rank	SMILES	Structure	Score
1	*O		0.665
2	*C		0.656
3	*OC		0.603
4	*CO		0.547
5	*OCC		0.471
6	*C=O		0.463
7	*CC#C		0.452
8	*CC		0.443

DeepFrag then generates a list of molecular fragments, sorted based on their numbers, and selects the top five fragments from each defined site. These fragments are downloaded in. mol2 format, resulting in a total of 20 molecules for further analysis.

The next step involves docking-based screening of all 20 molecules against the target protein. This screening process assesses the binding affinity and interactions of each molecule with the target site, providing valuable insights into their potential as drug candidates. In this study, the docking results revealed that site B of thymol exhibited greater suitability for subsequent optimization, as evidenced by its higher docking score compared to other sites.

Based on this insight, thymol was optimized by introducing an amine group and substituting an OH group after two carbon spacers. These modifications, inspired by successful drugs like the JAK inhibitor Tofacitinib, aim to enhance thymol's activity by incorporating electronegative atoms such as nitrogen, as illustrated in Figure 2.1

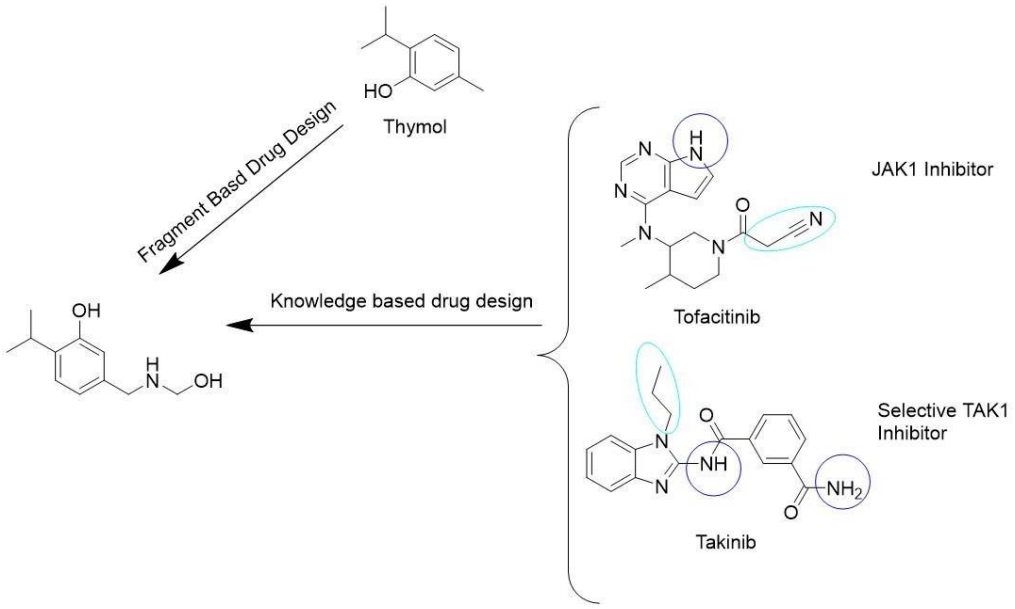


Fig.2.1 *Fragment-based and knowledge-based drug designed of Thymol*

4.1.3 Molecular docking studies

GOLD 5.2 is like having a supercharged molecular docking assistant. Think of it as a smart tool that uses a genetic algorithm to deeply explore how different molecules fit together, considering not just the shapes but also how flexible they can be. What's really neat is that it takes into account how proteins can move and even transforms flat 2D structures into realistic 3D ones. Plus, it's super important that it can shake loose those water molecules that get in the way of a good binding. In the world of drug discovery and understanding biological processes, having something as comprehensive as GOLD 5.2 can be a useful. (Barai et al. 18)

In GOLD 5.2, think of the docking score like how good a dance pair fits together. The higher the score, the better the match! But this score isn't just random; it's figured out by a smart computer program that looks at how well the chemical and protein get along. It measures the molecular weight and specific gravity, the number of bonds (or hydrogen bonds) the molecule forms with the protein, and its hydrophobicity (ability to bind water). By balancing all these things, the program gives a score that tells you how snugly the chemical and protein fit. So, if you want a strong

connection between your chemical and the protein, you want a high score – it's like finding the perfect fit to complete we complex for the effect we're aiming for. (Kumar et al. 1363; Pires et al. 4066)

The file that was generated as shown in Table 2.6, contains the optimal conformations of the ligands that were used to conduct docking tests. All these phytoconstituents were tested against the various targets of the RA viz. TAK1, JAK, COX and IL6**.** Among all these targets favorable results were obtained against the TAK1 and JAK as shown in Table 2.6.

Table 2.6 Reported findings of docking score from GOLD

Target (PDB ID)	**Ligands**	**GOLD SCORE**
5ML3 (COX)	Thymol	34.7412
	p-cymene	34.5102
	Linalool	30.808
	ϒ-Terpinene	35.1385
	Carvacrol	31.5485
	β-caryophyllene	30.9705
5V5N (TAK1)	Thymol	36.6036
	p-cymene	35.1706
	Linalool	35.5482
	ϒ-Terpinene	34.8963
	Carvacrol	35.3641
	β-caryophyllene	35.2257
6P9E (IL-6)	Thymol	35.3797
	p-cymene	34.3204
	Linalool	35.9378
	ϒ-Terpinene	35.9069
	Carvacrol	31.413
	β-caryophyllene	24.3071
7TEU (JAK)	Thymol	37.0596
	Designed Hit	49.4281
	p-cymene	32.2113
	Linalool	33.6652
	ϒ-Terpinene	36.0461
	Carvacrol	35.4426
	β-caryophyllene	34.8579

Based on the overall docking score, two proteins—5V5N (TAK1) and 7TEU (JAK)—were selected as the primary proteins. Co-crystallized ligands for each protein were identified using the protein database, and they were EDH and O6D, respectively. These co-crystallized ligands, water molecules, H atoms, and side chains were eliminated from the protein structure during the formation of proteins. The protein structure is simplified as a result of this elimination, making it simpler to see how the protein interacts with other molecules. Based on the location of the crystallized ligand, the docking site was selected.

4.1.4 ADMET studies

It may determine the drug molecule of the target molecule by utilizing pkCSM. This computation is based on the anticipated pharmacokinetic features, which depend on graph- based signatures as illustrated in Figure 3.6, and include absorption, distribution, metabolism, elimination, and toxicity. Predictive models are trained and tiny molecules are represented by pkCSM, which encodes the distance pattern between atoms. The pkCSM data is useful in identifying the characteristics of the molecules and determining whether or not they could be utilized in the development of a safe and efficient medication. This predictive data allowed to de identify the potential lead drug molecule candidates rapidly and cost effective making it critical resource in drug development. (Satpathy and Acharya 405) By inputting the drug molecule pkCSM generates the molecular properties like LogP, LogS, VD, P-gp inhibition and other factors, toxicity data as shown in Table 2.7.

Log S, also known as the logarithm of the partition coefficient (log P), is a measure of a compound's lipophilicity or hydrophobicity, which influences its absorption, distribution, and metabolism properties. Predicting log S is essential for assessing a compound's pharmacokinetic profile and its potential to penetrate biological membranes. Permeability

coefficients across monolayers of the human colon carcinoma cell line Caco-2, cultured on permeable supports, are commonly used to predict the absorption of orally administered drugs. When cultured as a monolayer, Caco-2 cells differentiate to form tight junctions between cells to serve as a model of paracellular movement of compounds across the monolayer. %Abs is the percentage of available compound absorbed across the intestinal barrier. Lipophilicity, molecular weight, and the presence of efflux transporters like P-glycoprotein can significantly impact %Abs.

Volume of distribution at steady state is a key pharmacokinetic parameter that describes the relationship between drug concentration measured in plasma or blood to the amount of drug in the body at equilibrium. Estimation of apparent VD is of utmost importance because it influences Cmax and half-life in plasma and target tissues, which in turn determines dose and dosing regimen in the clinicThe fraction of a drug that is unbound in plasma can exhibit pharmacological activity through interactions with targets such as proteins, enzymes, receptors, and channels. This information is crucial for building a pharmacokinetic model as it indicates the drug's efficacy.

Table 2.7 Reported findings from the pkCSM (ADMET studies) of Phyto constituent.

Ligand name	**log S**	**CACO2 Per**	**% ABS**	**VD**	**FRACTION UNBOUND**	**P-gp inhibition**	**CYP2D6 INH**	**CYP3A4 INH**	**CLR**	**RENAL OCT2**	**AMES toxicity**	**hERG 1 toxicity**	**LD 50**	**LOAEL**
thymol	-2.789	1.606	90.843	0.512	0.203	No	No	No	0.211	No	No	No	2.074	2.212
P-cymene	-4.081	1.527	93.544	0.697	0.159	No	No	No	0.239	No	No	No	1.827	2.328
linalool	-2.612	1.493	93.163	0.152	0.484	No	No	No	0.446	No	No	No	1.704	2.024
γ Terpinene	-3.941	1.414	96.219	0.412	0.42	No	No	No	0.217	No	No	No	1.766	2.394
Carvacrol	-2.789	1.606	90.843	0.512	0.203	No	No	No	0.207	No	No	No	2.074	2.212
β-caryophyllene	-5.555	1.423	94.845	0.652	0.263	No	No	No	1.088	No	No	No	1.617	1.416

Table 2.8 Reported findings from the pkCSM (ADMET studies) of new molecule

Ligand name	**log S**	**CACO2 Per**	**% ABS**	**VD**	**FRACTION UNBOUND**	**P-gp inhibition**	**CYP2D6 INH**	**CYP3A4 INH**	**CLR**	**RENAL OCT2**	**AMES toxicity**	**hERG 1 toxicity**	**LD 50**	**LOAEL**
New molecule	-1.923	1.191	93.103	0.825	0.699	No	No	No	1.02	No	No	No	2.408	1.65

P-glycoprotein (P-gp) actively transports a wide variety of chemically diverse compounds out of cells. Because it is crucial for drug absorption and excretion, inhibiting the p-glycoprotein transporter may have a number of negative medical effects as well as major effects on other drugs. Cytochrome P450 CYP2D6 and CYP3A4 is the most extensively characterized polymorphic drug-metabolizing enzyme. An essential enzyme for the body's detoxification process, cytochrome P450 is mostly located in the liver. The cytochrome P450 iso-forms induce the activation of certain medications and destroy many others. As a result, determining a compound's capacity to inhibit cytochrome P450 is critical. Renal clearance (CLr) plays an essential role in the elimination of drugs. The excretion of unchanged compounds by the kidney constitutes a major route in drug elimination and plays an important role in pharmacokinetics.

There are several subtypes of the renal organic cation transporter that mediate the transport of organic cations in the kidney. This transporter is crucial for the absorption or efflux of cationic medicines. Renal organic cation transporters inhibition has been linked to clinically significant medication interactions in the kidney. For this reason, knowing which medications could be inhibitors of the renal organic cation transporter is crucial. The Ames test is an experiment used to assess a substance's capacity to cause DNA alterations. The premise of this test is that, because DNA is chemically identical in all living things, bacteria can be utilized to more accurately identify substances that may cause cancer in humans. The potassium channel is encoded by hERG, which is responsible for the production of cardiac action potentials. Thus, abrupt cardiac death and QT prolongation are linked to hERG channel blockage. Because of this significant impact, evaluating the compounds' ability to block hERG is crucial.

One important stage in the drug discovery process is determining the acute toxicity, which is reported as the median lethal dose (LD50). The statistically determined dose that, when administered in an acute toxicity test, is the median lethal dose anticipated to result in the death of 50% of the treated animals in a specific amount of time is known as the LD50 value). Chronic studies are intended to identify the highest dose at which no negative effects are observed (NOAEL) and the lowest dose of a chemical that has an unfavourable impact (LOAEL). So, through these resulted properties ADMET data are trained, tested and predicted. Therefore, the molecules are important for the testing and future development of a possible rheumatoid arthritis medication.

Accordingly, the pkCSM property explorer was used in the current study to predict the toxicity of all of the obtained hits. This is indicated in Table 2.7 and aims to determine the solubility, P-glycoprotein I and II inhibitors, skin permeability, cytochrome P2D6, cytochrome P3A4, total clearance, renal OCT2 substrate, and AMES toxicity of the obtained hits.

Thymol was the most likely candidate out of all the virtual hits that were obtained because, as Table 2.7 illustrates, it had more desirable properties than any other drug, including solubility, P-glycoprotein I and II inhibitors, skin permeability, cytochrome P2D6, cytochrome P3A4, total clearance, renal OCT2 substrate, and AMES toxicity.

Consequently, Thymol may be viewed as a promising hit that can be further altered to provide a strong lead contender that can bind to the JAK. The newly developed hit (DH) underwent the same process based on these results, and as Table 2.8 shows, improvements were made in practically all metrics.

4.1.5 Molecular dynamics simulation

MD simulation start with the preparing a topology file of protein (large molecule) & ligand (small molecule). Which carried out by GROMACS software. In this software protein topology is generated but it can't generate the Ligand topology file so, for the generation of the ligand topology we use LigParGen server which online available.

In the simulation there are three major stapes 1st only protein run, 2nd protein ligand (complex) run which we obtain from the docking, 3rd Analysis of the result.

At the end of both simulations, we obtain graph files of RMSD (root mean square deviation), RMSF (root mean square fluctuation), H-bond, Radios of gyration, which is in. xvg format. Then in analysis we compare both the result's graph that which graph is more stable. Graph of RMSD, RMSF is shouldn't much fluctuated if is fluctuated the binding site in not much stable.

MD simulation of thymol and designed hit (DH) in complex with the target protein were carried out to understand the dynamics better. In order to generate topology file of protein OPLS-AA/L force field was used. An energy minimization was performed with GROMACS. 1 bar of pressure and 300 K were the settings for the NPT ensemble.

The steepest descent techniques were used to minimize the energy consumption of the system in 5,000 steps. NVT (number of particles, volume, and temperature) and NPT (number of particles, pressure, and temperature) equilibration was carried out for 100 picoseconds (ps) following the energy minimization. The simulation was run with time steps of 50 nanoseconds. then used the XMGRACE programmed to analyzed the result graph.

CHAPTER 5 Result and Discussion

5.1 De *Novo* Drug Design Enhanced by DeepFrag

Thymol exhibited promising docking results and demonstrated moderate binding interactions with the target proteins, namely JAK1. Through a systematic approach utilizing fragment-based drug design, thymol was strategically modified to enhance its efficacy, resulting in the development of a novel Hit candidate viz. the designed Hit (DH) is shown in Fig. 2.1 . The successful modification of thymol into a lead candidate marks a significant advancement in drug development targeting autoimmune and inflammatory diseases. By incorporating features from established drugs such as tofacitinib (a JAK1 inhibitor) into the structure of thymol, we aimed to capitalize on their known mechanisms of action while harnessing the inherent properties of thymol. The rationale behind incorporating features from existing drugs lies in their well- characterized pharmacological profiles and clinical efficacy. Tofacitinib, for instance, has shown remarkable success in the treatment of rheumatoid arthritis by selectively inhibiting JAK1, thereby modulating inflammatory responses. By integrating these features into the structure of thymol, we aimed to create a targeted therapeutic agent capable of modulating the JAK pathways. This approach not only enhances the potential efficacy of the lead candidate but also addresses the limitations often associated with single-target therapies, such as drug resistance and incomplete disease management.

Furthermore, the use of fragment-based drug design allowed for precise modifications to be made to thymol, optimizing its pharmacokinetic and pharmacodynamic properties while maintaining its favorable safety profile. This iterative design process enables the fine-tuning of molecular interactions, ultimately leading to the development of a lead candidate with improved potency and selectivity.

In conclusion, the successful modification of thymol into a lead candidate with enhanced activity against JAK represents a promising advancement in the field of drug discovery for autoimmune and inflammatory diseases. Further preclinical and clinical studies are warranted to validate the efficacy and safety of this novel therapeutic agent, with the potential to address unmet medical needs and improve patient outcomes

5.2 Molecular docking studies

Standard co-crystallized ligand of the TAK1-EDH complex and JAK-O6D complex molecular binding interaction, as illustrated in Fig. 3.1, 3.2, 3.3, 3.4. The pi sulphur interaction between thymol and MET A:104, as depicted in Fig. 3.3, is comparable to the binding interaction of the standard ligand EDH, as depicted in Fig. 3.1. Additionally, it demonstrated an alkyl bond with Leu A:163, which is comparable to the conventional ligand EDH. Consequently, there is a reasonable chance that thymol will function as an efficient TAK1 inhibitor. As seen in Fig. 3.4, Thymol also exhibited hydrogen bond interaction with TYR A:972, which is comparable to the JAK-O6D complex as demonstrated in Fig. 3.2, suggesting its inhibitory ability against the JAK.

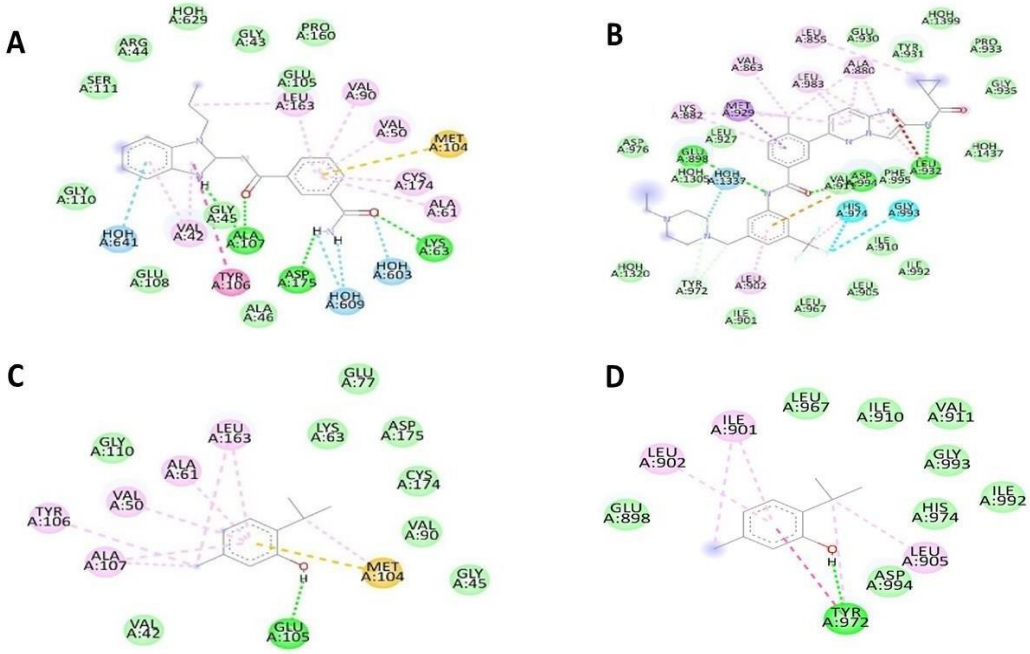


Fig. 3.1 *2D-Interaction of standard co-crystallized ligand (EDH) with the TAK1 protein (PDB ID:5V5N), (3.2) 2D-Interaction of standard co-crystallized ligand (O6D) with the JAK protein (PDB ID:7TEU) and (3.3) 2D- Interaction of thymol after performing docking with the TAK1 protein (PDB ID:5V5N) and (3.4) 2D-Interaction of thymol after performing docking with the JAK protein (PDB ID: 7TEU)*

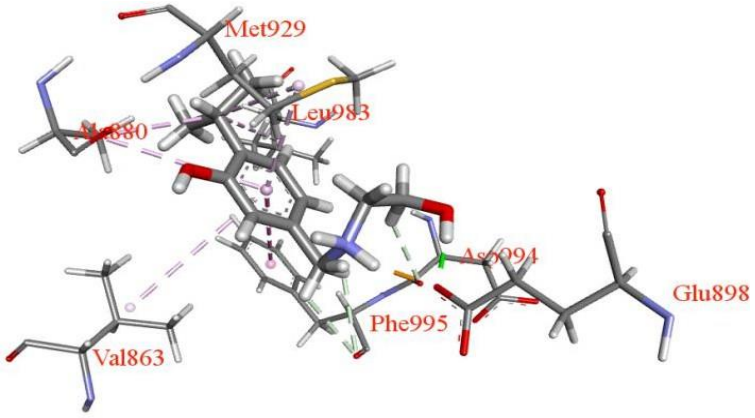


Fig.3.5 *The molecular docking analysis of the Designed Hit (DH) with the JAK protein (PDB ID: 7TEU) demonstrates an enhanced binding affinity compared to the original interaction of the Thymol with the JAK protein. This improvement is supported by the binding interactions observed with specific amino acids, namely Val 863, Ala 880, and Asp 994, which closely resemble the binding pattern of the co-crystallized ligand O6D of JAK protein.*

However, Thymol consistently demonstrated superior results against JAK. Consequently, further investigations in this study were focused on optimizing Thymol against JAK by exploring various fragments at different positions. Leveraging fragment-based and knowledge-based drug design strategies, a novel compound was designed, as depicted in the Fig. 2.2. This newly designed compound exhibited enhanced docking interactions as shown in Fig. 3.5. and scored 49.4281 against JAK, surpassing both Thymol and the co-crystallized ligand. This suggests that the new molecule possesses greater inhibitory potential against JAK compared to Thymol.

5.3 ADMET studies

The target molecule's drug molecule can be determined by utilising pkCSM. This computation is based on the anticipated pharmacokinetic characteristics, which include toxicity, distribution, metabolism, absorption, and elimination. These features are dependent on graph-based signatures, as illustrated in Figure 3.6. Predictive models are trained and tiny molecules are represented by pkCSM, which encodes the distance pattern between atoms.

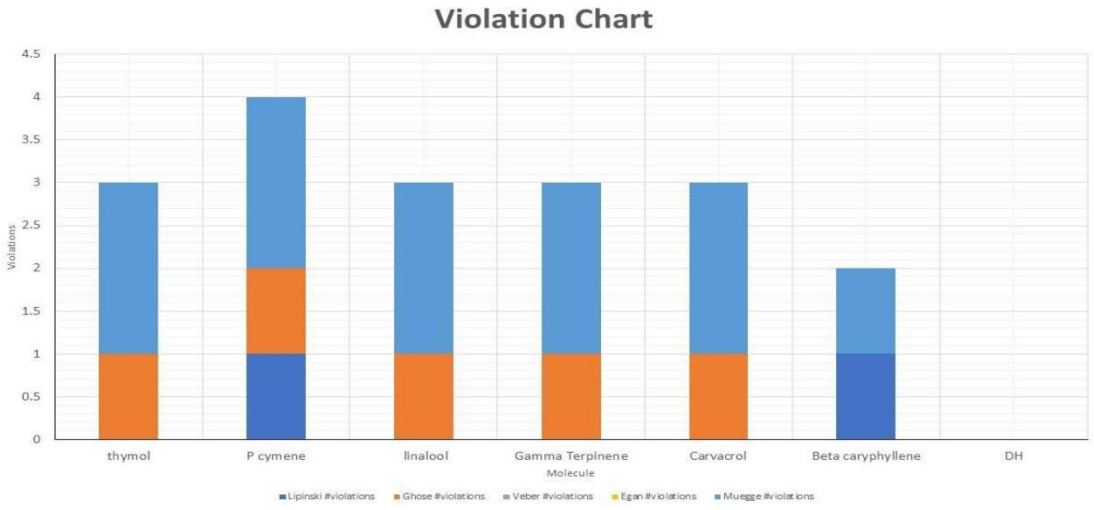


Fig. 3.6 *Result of the hits against different violations for the rules of draggability and drug-likeness.*

The pkCSM data is useful in identifying the characteristics of the molecules and determining whether or not they could be utilized in the development of a safe and efficient medication. This predictive data allowed to de identify the potential lead drug.

molecule candidates rapidly and cost effective making it critical resource in drug development. By inputting the drug molecule pkCSM generates the molecular properties like LogP, LogS, VD, P-gp inhibition and other factors, toxicity data as shown in Table 2.7. So through these resulted properties ADMET data are trained, tested and predicted. As such the molecules are prominent for the further development of and testing of the potential treatment for the rheumatoid arthritis.

Accordingly, the pkCSM property explorer was used in the current study to predict the toxicity of all of the obtained hits. This is indicated in Table 2.7 and aims to determine the solubility, P-glycoprotein I and II inhibitors, skin permeability, cytochrome P2D6, cytochrome P3A4, total clearance, renal OCT2 substrate, and AMES toxicity of the obtained hits.

Thymol was the most likely candidate out of all the virtual hits that were obtained because, as Table 2.7 illustrates, it had more desirable properties than any other drug, including solubility, P-glycoprotein I and II inhibitors, skin permeability, cytochrome P2D6, cytochrome P3A4, total clearance, renal OCT2 substrate, and AMES toxicity. Consequently, Thymol may be viewed as a promising hit that can be further altered to provide a strong lead contender that can bind to the JAK. Based on these findings, the newly designed hit (DH) underwent the same procedure, demonstrating improvements across almost all parameters, as illustrated in Table 2.8.

5.4 Molecular dynamics simulation

Using a 50 ns molecular dynamic simulation, the trajectory analysis for both targets is displayed in Figures 3.7 through 3.8. During the MD simulation process, the average distance of complexes between atoms in the proteins is used to calculate parameters like RMSD. In this work, the Thymol-JAK (7TEU) complex was found to be stable after 40 ns, as shown in Fig. 3.8, but the overall RMSD of the Thymol- TAK1 (5V5N) complex displayed extensive variation till 50 ns, as shown in Fig. 3.7.

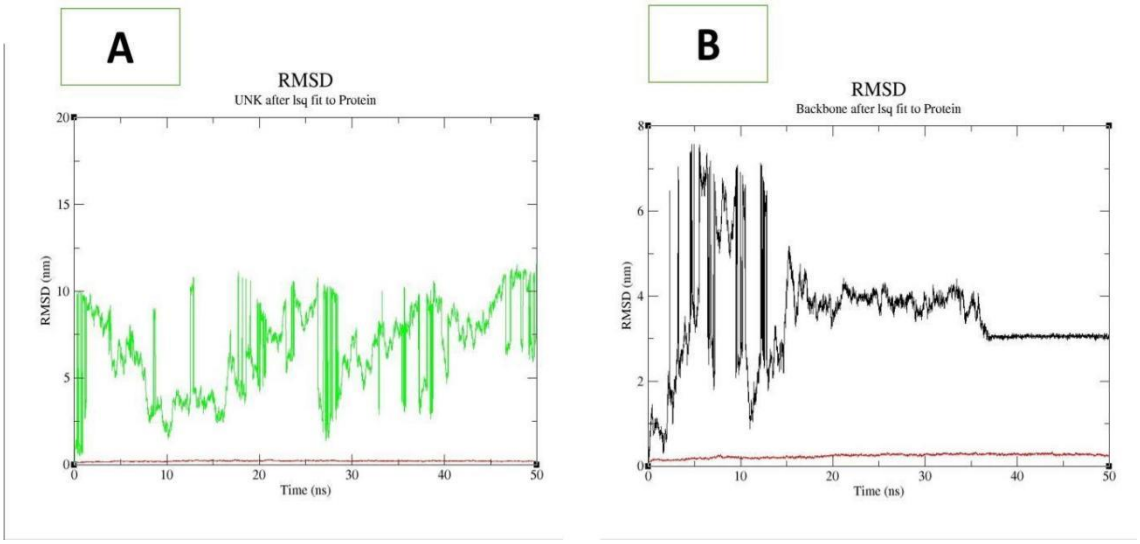
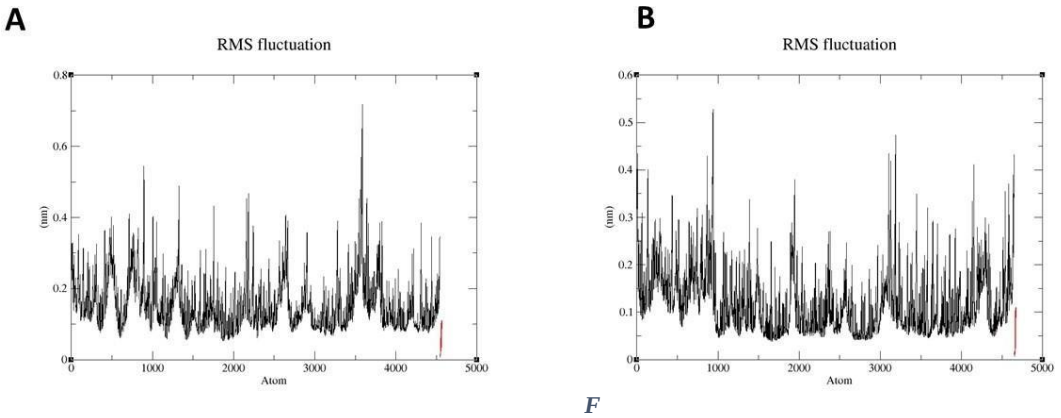


Fig.3.7 *Molecular dynamic simulation of protein (5V5N) – ligand (Thymol). RMSD plot for protein – ligand complex during 50 ns of molecular dynamics simulation. Protein is shown in the red color and Complex in the Green color. (3.8) Molecular dynamic simulation of protein (7TEU) – ligand (Thymol). RMSD plot for protein – ligand complex during 50 ns of molecular dynamics simulation. Protein is shown in the red color and Complex in the Black color.*

Throughout the simulation period, the RMSF parameter determines the flexibility property of the protein and produces the flexibility of each individual amino acid. Every atom's fluctuation over the entire simulation is provided via RMSF. RMSF was calculated of 5V5N Fig.3.9 and 7TEU Fig.3.10 and fluctuations obtained was < 0.55 and < 0.5 respectively. By comparing both the complexes, Thymol-JAK (7TEU) showed the best result as it had less deviation (Costa et al. 1151; Shaw et al.; Parekh et al. 100009; Chaube and Bhatt 1895; Scherer et al. 102400).



F

ig.3.9 *RMSF graph of TAK1-Thymol complex (PDB:5V5N) and (3.10) JAK-Thymol complex (PDB 7TEU)*

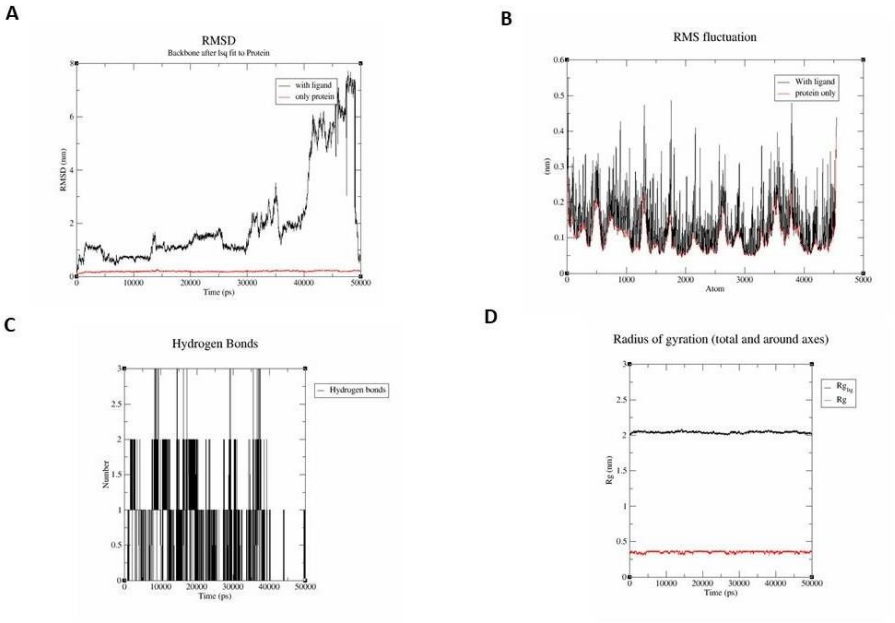


Fig. 3.11 *Molecular dynamic simulation of protein (7TEU) – Ligand {Designed Hit (DH)}. RMSD plot for protein– ligand complex during 50 ns of molecular dynamics simulation. Protein is shown in the red color and Complex in the Black color. (3.12) RMSF plot for – Ligand {Designed Hit (DH)}and of protein (7TEU). Protein is shown in the red color and Complex in the Black color (3.13) Hydrogen bond plot for protein – ligand complex (3.14) Radios of gyration plot for protein – ligand complex*

Molecular dynamics simulation was conducted for the newly designed hit (DH) over a period of 50 ns using the same method. The simulation results are illustrated in Fig. 3.11 where Fig. 3.11 displays the RMSD of the complex formed by the new molecule and the JAK protein. Fig. 3.12, 3.13, and 3.14 present the RMSF, hydrogen bond interactions, and radius of gyration, respectively.

CHAPTER 6 Conclusion

Autoimmunization is the major problem associated with the Rheumatoid arthritis disease where older people are most affected.

In this work, efforts were made to discover novel therapeutic targets for the drug development of the rheumatoid arthritis as millions of people are suffering from these diseases every year, hence this study predicts the potential inhibitory action of 6 bioactive constituents of *Thymus linearis* against inhibitor of rheumatoid arthritis using computational models. The GOLD 5.2 docking score takes into account size, shape, hydrogen binding, and hydrophobic interactions when assessing how molecules interact with the target protein. Thymol demonstrated good binding score with the TAK1 and JAK protein, indicating a plausible protest for being low-risk, efficacious treatment for arthritis. Additionally, the MD stimulation outcome by GROMACS software where thymol showed the better interaction with JAK when compared with other inhibitory protein. It was determined that thymol might be a viable treatment option for rheumatoid arthritis based on the data that was acquired from ADMET using pkCAM software.

Thymol has demonstrated a satisfactory pharmacokinetic profile, according to the Veber, Ghose, Lipinski, and Eggen rules with only one violation as shown in Table 4.1, and indicating that it may be a potential hit compound for a medication. Additionally, the Thymol was shown to be non-toxic in the toxicity analysis conducted using the pkCAM property explorer.

Table 4.1 The Veber, Ghose, Lipinski, and Egan rules with violations

Molecule	**Lipinski #Violations**	**Ghose #Violations**	**Veber #Violations**	**Egan #Violations**	**Muegge #Violations**
Thymol	0	1	0	0	2
Designed Hit (DH)	0	0	0	0	0
Carvacrol	1	1	0	0	2
p-cymene	0	1	0	0	2
Gamma-terpinene	0	1	0	0	2
Beta-caryophyllene	0	1	0	2	2
Linalool	1	0	0	1	1

Furthermore, Thymol underwent optimization using a *de novo* drug design approach, with DeepFrag software aiding in the identification of effective substitutions. Additionally, a knowledge-based drug design was employed to develop a new designed hit (DH) compound for potential exploration against JAK in the treatment of RA.

CHAPTER 7 Future Perspectives

In summary, our findings suggest that Thymus Linearis could be investigated further for its potential in treating RA. To advance therapeutic options for rheumatoid arthritis, it is recommended that these compounds be extracted from the plant and subjected to additional evaluation against JAK. Thymol, being a simple compound, was thus transformed in this study into a suitable hit (DH) compound for synthesis and subsequent evaluation against JAK as a potential treatment for RA.

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