Systematic evaluation of natural compounds of *Colchicum Luteum* and *Trachyspermum Ammi* to identify the potential targets for Rheumatoid Arthritis.

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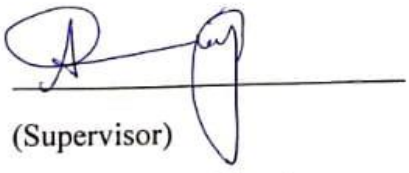

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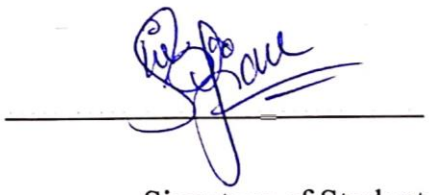
I certify that this research work titled "Systematic evaluation of natural compounds of *Colchicum Luteum* and *Trachyspermum Ammi* to identify the potential targets for Rheumatoid Arthritis" is my own work. He work has not been presented elsewhere for assessment. The material that has been used from other sources have been properly acknowledge or referred.



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First of all, thanks to **ALLAH SWT**, Who is the most Beneficent and the most Merciful, Whose blessings are abandon and favors are unlimited. In addition, may peace and salutation be given to the **Prophet Muhammad** ﷺ who has taken all human beings from the darkness of ignorance to the light of knowledge.

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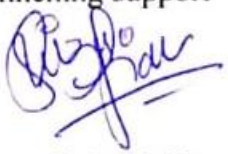

Shiza Sufian

Dedicated to my Parents

the two pillars of my life who have provided me with the best they could at every step of my life.

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ABSTRACT

Rheumatoid Arthritis is a chronic inflammatory disease effecting 0.5 – 1% of the population. Rheumatoid is more common among females as compared to males. It is a progressive disease and there is no cure yet. The current treatment of Rheumatoid Arthritis includes the use commercial drugs that are majorly classified as NSAIDs, DMARDs, Glucocorticoids and Biologics. *Trachyspermum Ammi* and *Colchicum Luteum* are the medicinal plants exhibiting anti-inflammatory and anti-oxidant activity. The active phytochemicals of these plants can be used for the inhibition of various inflammatory pathways to slow down the progression of disease without causing any serious side effects as compared to the synthetic commercial drugs. The shortlisted phytochemicals of both the plants showed excellent physical and chemical characteristics to be used as anti-arthritic agent. The comparative in-silico docking analysis of shortlisted phytochemicals and commercial drugs to the common Rheumatoid Arthritis targets showed that various phytochemicals binding affinity was better than the commercial drugs resulting a good target ligand interaction. This analysis concludes that the shortlisted active phytochemicals having better affinity values could be considered for further studies as compared to the commercial drugs available for Rheumatoid Arthritis.

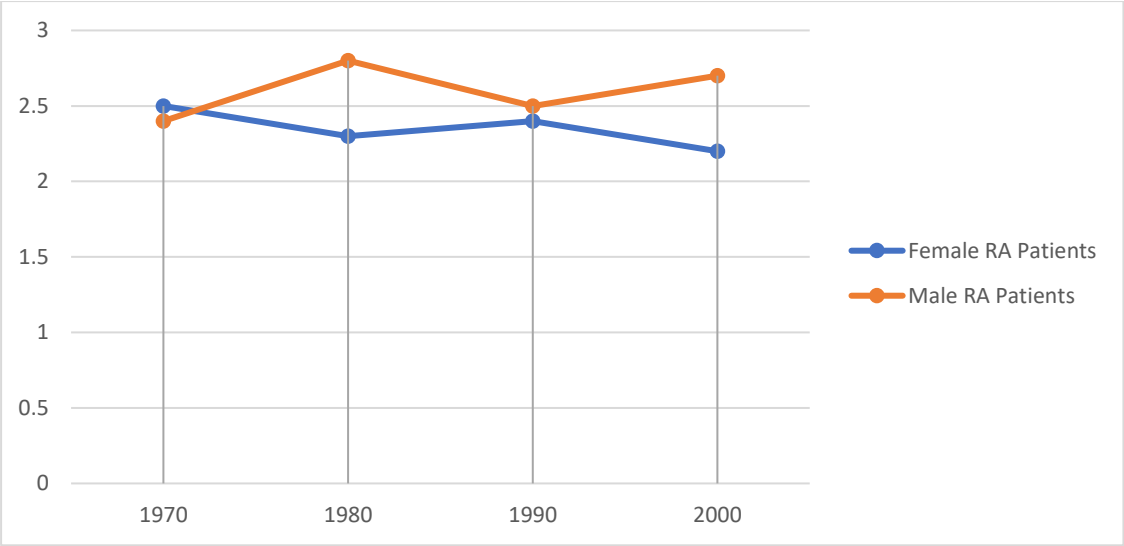
1. INTRODUCTION: Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease associated with progressive inflammation of joints. It is considered as a very important cause of disabilities. Initially, rheumatoid arthritis affects small joints in arms and legs but the inflammation progress and affects synovial tissues (Firestein, 2003). It affects 0.5 – 1% of the population around the world (Marra et al., 2011). In recent studies, the cause of rheumatoid arthritis has been determined to be based on genetics and epigenetics. But along with the genetics, environment also plays an important. Many factors are studied that can be associated with rheumatoid arthritis. These factors may include smoking, socioeconomic status, birthweight, and alcohol consumption. It emphasize on the complex interaction between the genetic and the environmental factors that triggers the autoimmune response which in-return develops rheumatoid arthritis (Liao et al., 2009). The course of the disease is also associated with other aspects which includes gender, age, racial and socioeconomic reasons. These issues have been reported to cause an immense burden on the country's financial and economic resources along with the increasing burden on the health departments leading to a drop in the quality of health-care system. This issue also affects the health of the patient life style in general (Wolfe et al., 1986). Various studies have been done to study the related comorbidities to RA. The inflammation of RA progresses with time and this increase in the infection as suppresses immune system. Septic Arthritis is known to be a cause of great concern. There is a higher risk of other infections for RA patients as compared to the normal population (Wolfe et al., 1986). The mortality rate is also increased about by 2 folds in RA patients as compared to the general population. This increase also depends upon the other factors which include age, and related comorbidities (Gonzalez et al., 2007).

1.1 Epidemiology of Rheumatoid Arthritis

Increase in the mortality rate of Rheumatoid Arthritis has been reported. The main reason behind this increase is the number of comorbidities linked to the disease and also the presence of the Rheumatoid Factor has seen to be another cause of increases in the mortality gap among the patients of RA (Myasoedova et al., 2010). There is no evidence to whether the survival has improved. Some studies have shown to have the improved mortality rate over the years. This could be the because of the early diagnosis of RA and the new advance medication (Gonzalez et al., 2007).

A study conducted by Gonzalez and the colleagues (2007) in a population in Minnesota to understand the mortality rate. They calculated the rate over the period from 1955 to 2000 depending on the age, sex and the duration of the disease of patients and concluded that the mortality rate stayed persisted over the few decades and no apparent change was seen both among the males and the females but on comparing the mortality rate with the general population, and it was considerably higher which is an alarming situation (Gonzalez et al., 2007).



Graph 1: Data of RA patients based on gender in Minnesota US white population over the decades. Modified from (1970 - 2000) (Gonzalez et al., 2007)

Another study was done by the Widdifield and colleagues (2014) in Ontario, Canada regarding the epidemiology of RA among the general population from 1996 – 2010. This research was also age and sex based. They concluded that the prevalence and the mortality trend of RA patients increased with time. It was also noticed from the results that the prevalence of the disease increased with age while the incidence was slowly decreasing (Widdifield et al., 2014).

The prevalence of RA has been reported to be high in west i.e., in Europe and America with higher incidence rate in females as compared to the studies conducted in the developing countries (Tobón et al., 2010). World Health Organization (WHO) and the International League Against Rheumatism collected data from different countries in Asia like Philippines and China. And on conducting a meta-study on the population of India and Pakistan to understand the rate pf prevalence in these countries as both the countries also share the ethnic identity gave an insight at the estimation of Rheumatoid Arthritis. According to the reports, prevalence is almost same in both the countries but still representing the general idea of prevalence rate higher in Europe and America as compared to South Asia and Far East (Akhter et al., 2011).

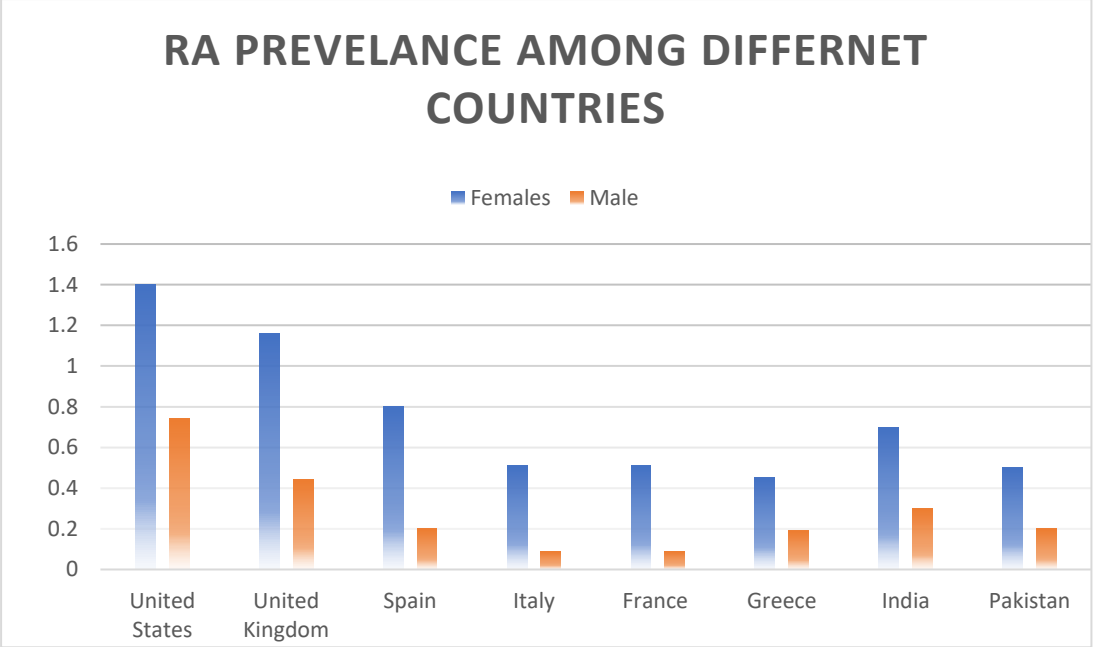


Figure 2 Prevalence of Rheumatoid Arthritis in various countries based on gender. Modified from (Akhter et al., 2011; Tobón et al., 2010)

From various studies the higher prevalence among females is seen irrespective of the demographics by two folds as compared to male. Males have approximately 1.7% and females have 3.6% of the chance of developing RA during the course of their life (Crowson et al., 2011) and both the environmental and genetic factors contribute their role in the occurrence of the disease (Alamanos et al., 2005).

1.2 Pathogenesis of Rheumatoid Arthritis

The pathogenesis of RA is characterized by the auto-immune inflammation in the synovial fluid of the joints, bone erosion, cartilage erosion, auto-antibody production (McInnes et al., 2011). The anti–citrullinated protein antibody (ACPA) plays an important role in pathogenesis of the disease (Kurowska et al., 2017).

Basically, the clinical presentation of the disease includes pain and swelling in the joints and also the nodules around the affected area. Hand involvement is early in the disease tends to affect metacarpal, phalangeal and proximal interphalangeal joints.

There is also extra-articular involvement but as the disease progresses it starts to worsen the condition of the patient as they start to feel stiffness in joints and pain while moving.

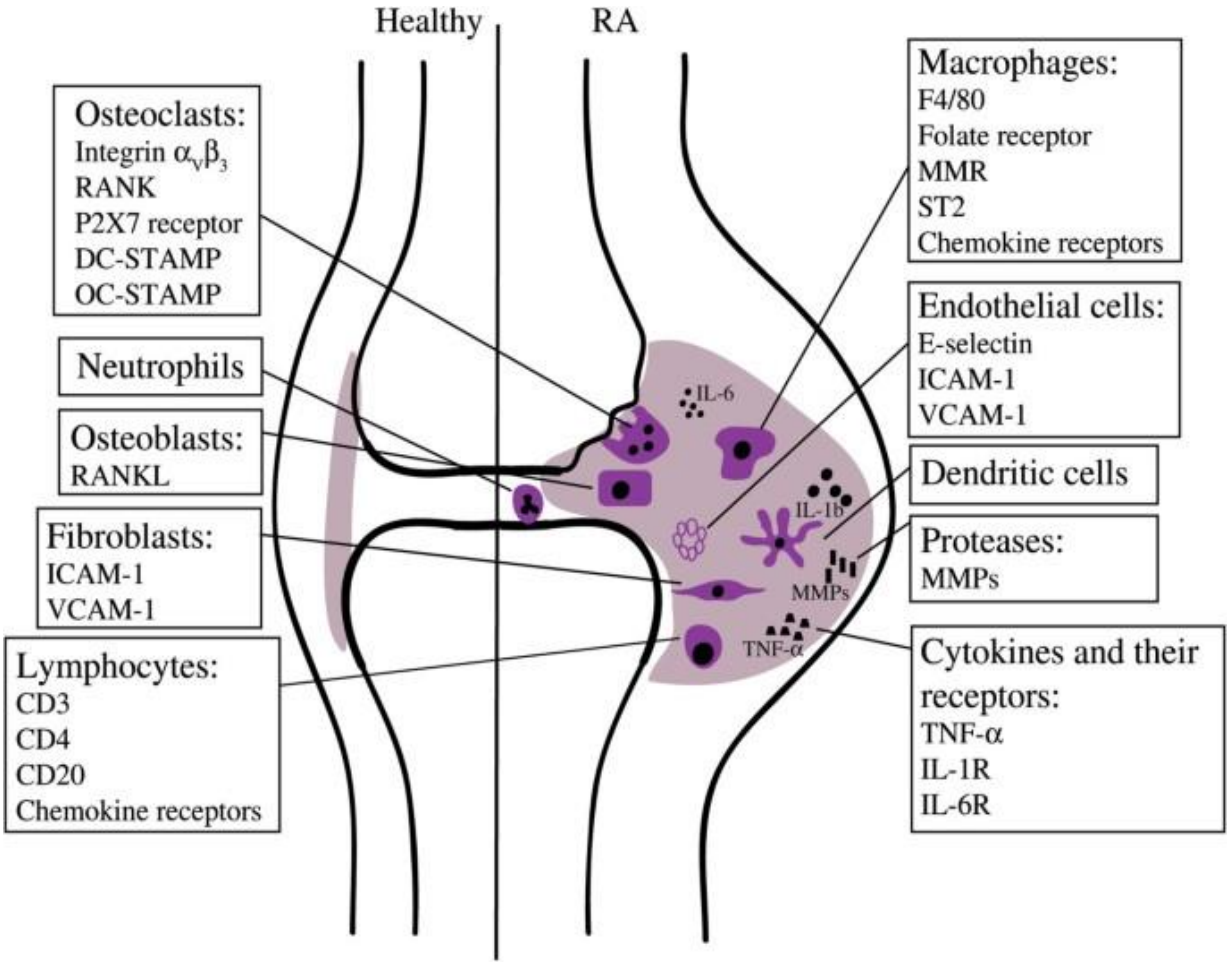


Figure 1 Schematic View of a healthy and an inflamed RA joint showing the release of cytokines involved in pathogenesis (Put et al., 2014).

In the normal healthy joint, synovial fluid is present in the joint as lubrication and supply the nutrients to the area. In RA the inflammation of the synovium have the sign of 'Itis' which results in pain and swelling leading to cartilage and bone erosion. Another feature in the joints of the Ra patient is the angiogenesis. The synovial membrane that is made of cells known as fibro blast like synovial sites and these are considered very important in the pathogenesis of RA. The macrophages present in the inflamed joint secret cytokines such as TNF-alpha Interleukin 1 (IL-1) and Interleukin

6 (IL-6). These cytokines stimulate the fibroblasts like synovial cells which essentially leads to bone and cartilage degradation (Srirangan et al., 2010).

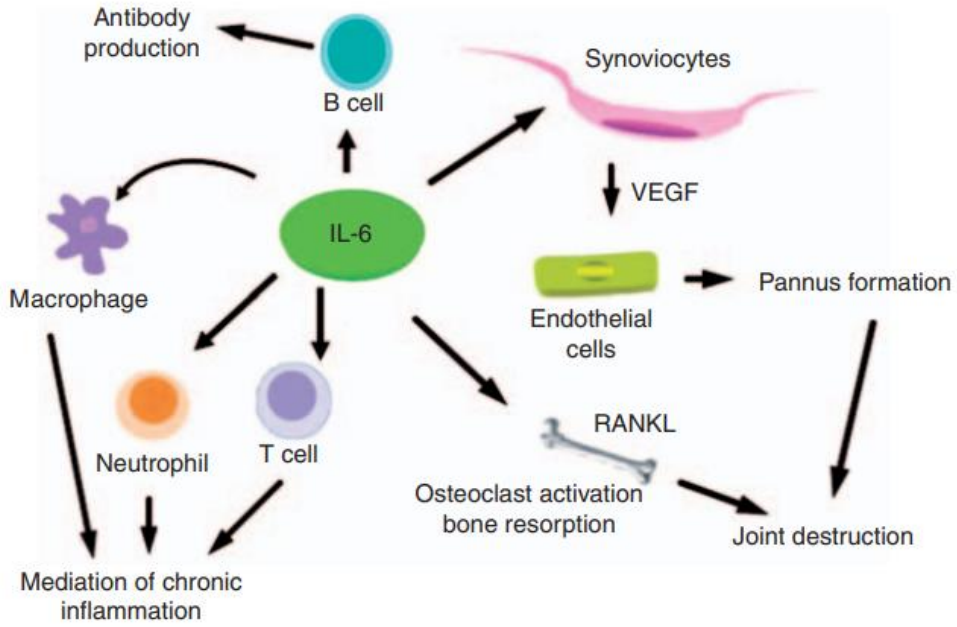


Figure 2 The inflammation activity stimulated by IL-6 resulting in Joint destruction (Srirangan et al., 2010).

Another important feature of the fibro blast like synovial site is that when it is stimulated they tend to migrate from joint to joint in the body leading to symmetrical arthritis in rheumatoid Arthritis. T-cells and B-cells which make up the large portion of the immune system are also involved in the process contributing to the inflammation. T-cells promote inflammation and also secret IL-17 also contributing to bone erosion (Put et al., 2014).

The activation of pro-inflammatory cytokine stimulated dendritic cells through extraneous species and autologous proteins that marks the activation of the innate immunity which is considered to be the very earliest even in the pathogenesis of RA.

In the adaptive immunity response, the IL-6 plays role in the pathophysiology of the disease. The IgG, IGM and ACPAs concentration increases (Kurowska et al., 2017). IL-6 indues the depletion of B-cells by stimulating the differentiation for the

production of antibodies. IL-6 also stimulated the differentiation of T-cells resulting in the production of IL-17 found mostly in the autoimmune diseases confirming its role in the pathophysiology of the adaptive immunity of RA (Srirangan et al., 2010). While B-lymphocytes are involved [in the activation of the RF, autoantibody production and the anti-cyclic citrullinated peptides (CCP) which aid in the formation of large immune complexes and also contributing to the secretion of the cytokines like TNF-alpha. The cytokine secretion in turn activates the production of Matrix metalloproteinases (MMPs) that causes pannus formation resulting in the bone erosion (Smolen et al., 2007). The systemic steps of the RA inflammation is shown in figure 3.

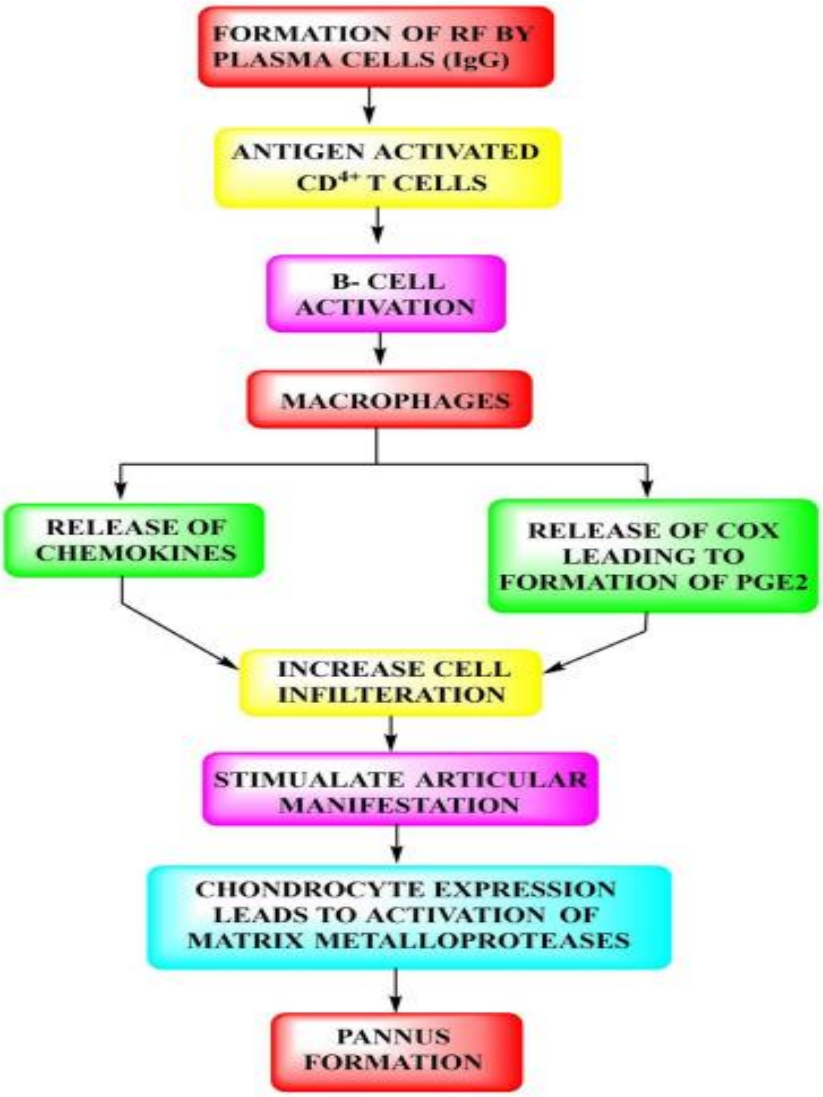


Figure 3 schematic View of the Pathogenesis of Rheumatoid Arthritis (Singh et al., 2020)

1.3 Etiology of Rheumatoid Arthritis

The exact cause of RA is unknown. But many factors are reported to play role e.g. sex, age, gender, ethnicity, hormonal levels, alcohol consumption, weight, smoking and diet (Alamanos et al., 2005).

1.4 Environmental Factors

There are various factors that have the tendency to modify the risk of the development of RA. The most studied ones are the smoking, diet, weight, and the socioeconomic status.

- Smoking

The smoking is known to be a great risk for causing RA. It is associated with an increased risk of the positive ACPA which eventually also leads positive RF as well (Liao et al., 2009). When exposed to the Tobacco, it simulates citrullination which leading to the production of ACPA; antibodies to citrullinated peptide. This activates the activity of cytokines having the strong tendency of causing inflammation leading to the development of RA (Van der Helm-van Mil et al., 2007). This is an ideal example of the environment-gene interaction.

- Alcohol consumption

Alcohol consumption has seen to have inverse relation with the possibility of developing of RA (Maxwell, 2010). The pathways were studied concluding the tendency of alcohol consumption to decline the intensity of immune response down regulating the production of pro-inflammatory cytokines (Hazes, 1990).

- Hormonal Risk

There is an evidence of hormonal levels as a risk factor of developing RA as the incidence rate of RA is higher in females as compared to males (Alpízar-Rodríguez & Finckh, 2017). A disturbed menstrual cycle increases the likelihood of developing the disease (Østensen et al., 1983).

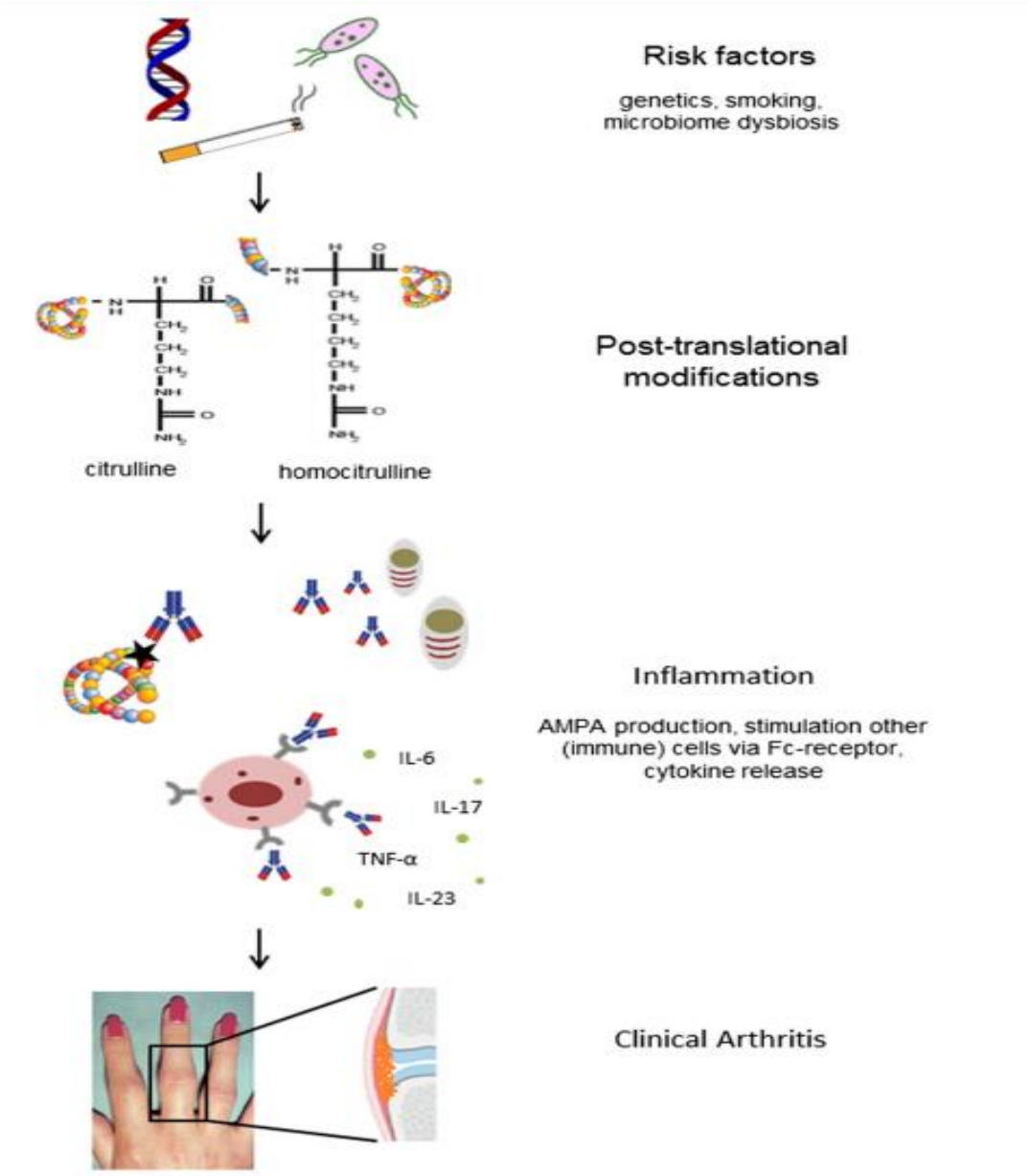


Figure 4 (Derksen et al., 2017)

1.4.1 Genetic Factors

HLA-DRB1 allele plays an important role when comes to the genetic factors. It shares the epitope (Scally et al., 2013)

1.5 Treatment

Presently the treatment options for Ra includes non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) and biologic (Pincus et al., 1999).

1.5.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

There are various NSAIDs that are normally used for inflammation that includes aspirin, Advil, Motrin, ibuprofen, celecoxib. These medications are also used in the earlie9r stages of RA. The immense use of this class of drug is based on their three important properties, they are antipyretic, analgesic and are anti-inflammatory. But there are numerous side effects due to which they are less used when the RA progress (Rao & Reddy, 2004).

The main mechanism of action for NSAIDs is that they inhibit cyclooxygenase enzyme (COX1 and COX2). COX1 enzymes help in the conversion of arachidonic acid into prostaglandins. Prostaglandins help in the house keeping functions of the body e.g., it regulates the mucosal lining of the gut and also helps in the regulation of clotting activity in the blood while COX2 turns on and generates select prostaglandins in response to only when needed particularly in response to inflammation COX2 generates the prostaglandins that intensify the symptoms of inflammation (Cashman, 1996).

1.5.2 Disease modifying anti-rheumatic drugs (DMARDs)

DMARDs are widely used for the treatment of Arthritis. The most popular DMARD is ‘Methotrexate’. It is the first choice drug (Caporali et al., 2008). Methotrexate is prescribed in low doses because it is also known to have some common side effects in the patients are Gastric Irritation and Stomatitis. Methotrexate causes a

severe bone marrow depression because of which it cause Pancytopenia 9Sosin & Handa, 2003).

The other important adverse effect is Hepatotoxicity caused by the fibrosis or cirrhosis of liver. Patients with kidney disease, chronic hepatitis, heavy alcohol consumption, Diabetes Mellitus and obese are at the risk of developing hepatotoxicity and also causes hypersensitivity (Valentino et al., 2014).

1.5.3 Biologics

Biologics are widely used now a days for the treatment of diseases. They are derived from biological processes rather than manufactured chemically (Curtis & Singh, 2011). Biologics are broadly characterized in to two groups: Tumor Necrosis Factor -- alpha (TNF) and the others. The drug for the biologics includes rituximab, Tocilizumab, Anakinra, cytokine antagonist, kinase inhibitors and abatacept (Nikiphoru et al., 2017). This treatment is very costly and includes risk of bacterial infection, and failure to keep the response for longer duration (Divonne et al., 2017).

1.6 Objectives

- To determine phytochemicals of *T. Ammi* and *C. Luteum* and shortlisting of active compounds.
- Identification of targets for the drug reposition of RA.
- In-silico evaluation of the shortlisted phytochemicals and commercially available drugs against RA targets.
- Comparative molecular docking analysis and ligand protein interactions

2. LITERATURE REVIEW

Oxidation reduction reaction is important for stable immune system. any imbalance in the reaction leads to the progression in RA. The imbalance is between the pro-oxidative compounds and the anti-oxidative compounds and normally the increase in the reactive oxygen species causes malfunctioning of the immune system (Filippin et al., 2008). It increases the risk of activating pathways that plays an important role in the pathophysiology of RA as discussed earlier. This reaction can be activated in response to drugs, stress, diet, and hormonal imbalances (Phull et al., 2018). In normal conditions, ROS play's role in safeguarding the biological system from the pathogens and also aid in the regeneration but when the functioning alters it can cause many diseases e.g., neurodegenerative, inflammatory disease and even cardiovascular (Abbas & Monireh, 2008). The reactive oxygen and nitrogen species are collectively involved in ROS. One is the secondary messenger while the other is a pathologic mediator further having two classification i.e., radicals (Superoxide, Hydroxyl Radical and NO), and Non-radicals (Hydrogen peroxide) (Griffiths, 2005). ROS in general play's its role as a secondary messenger to activate the genes that are related to the inflammatory response (Kohchi et al., 2009). It was initially reported that ROS activates NF-κB which is responsible for activating Tumor Necrosis Factor- alpha (TNF- α) and Interleukin 1 Beta (IL-1β) further activating the inflammatory cascade (Li et al., 2018).

This redox reaction activation eventually leads to apoptosis of cells. In mouse models the ROS reaction was manipulated which reported the role of ROS in RA leading to a complex inflammatory process which consists of various pro-inflammatory cytokines and signaling pathway playing an important role in the progression of the disease (Filippin et al., 2008).

1.7 Signaling Pathways Involved in Rheumatoid Arthritis

1.7.1 Jak and Stat Pathway

This pathway is activated by the mediated cytokine signals which can in the form of Interferon, IL or even Growth Factors (GF). The JAK STAT components in the plasma membrane having some of its part inside the cell and some of the component lies out of the cell. The outside of the cell components have cytokine Receptors showing the elevated levels of different cytokines including TNF-α, IL-1β, IL-6 and IL-17 specially among the patients of RA (Walker & Smith, 2005). Along with the receptor, he intracellular part of the component lies two proteins known as Janus Kinase (JAK) associated with the receptors (Malemund, 2018). On binding of the cytokine, phosphorylation process initiates leading to the formation of the phosphorylates tyrosine kinase mediating the docking of the STAT protein. This forms the STAT dimer playing role in the gene expression involved in the inflammatory cascade (Kasperkovitz et al., 2020).

In RA patients, the inflammatory process can be halted by using the JAK inhibitors. JAK1, JAK2 and JAK3 proteins are involved in the process (Ciaobanu et al., 2020). Commercial drugs available for the JAK STAT pathway inhibition are Barcitnib, Tofacitnib, and Upadacitnib. Barcitnib is an inhibitor for JAK1 and JAK2. Barcitumab is also a reversible inhibitor downregulating the IL7, IL15, IL21, IL6, IFN-alpha, and IFN-beta preventing the inflammatory signaling pathway and is prescribed to RA patients. The drug was approved in 2017 by the European Union. Tofacitinib is Food and Drug Authority (FDA) approved inhibitor of both JAK1 and JAK3 and is also prescribed to the RA patients while the Upadacitinib is an approved inhibitor of JAK1 selectively (Burja et al., 2020; Simon et al., 2020).

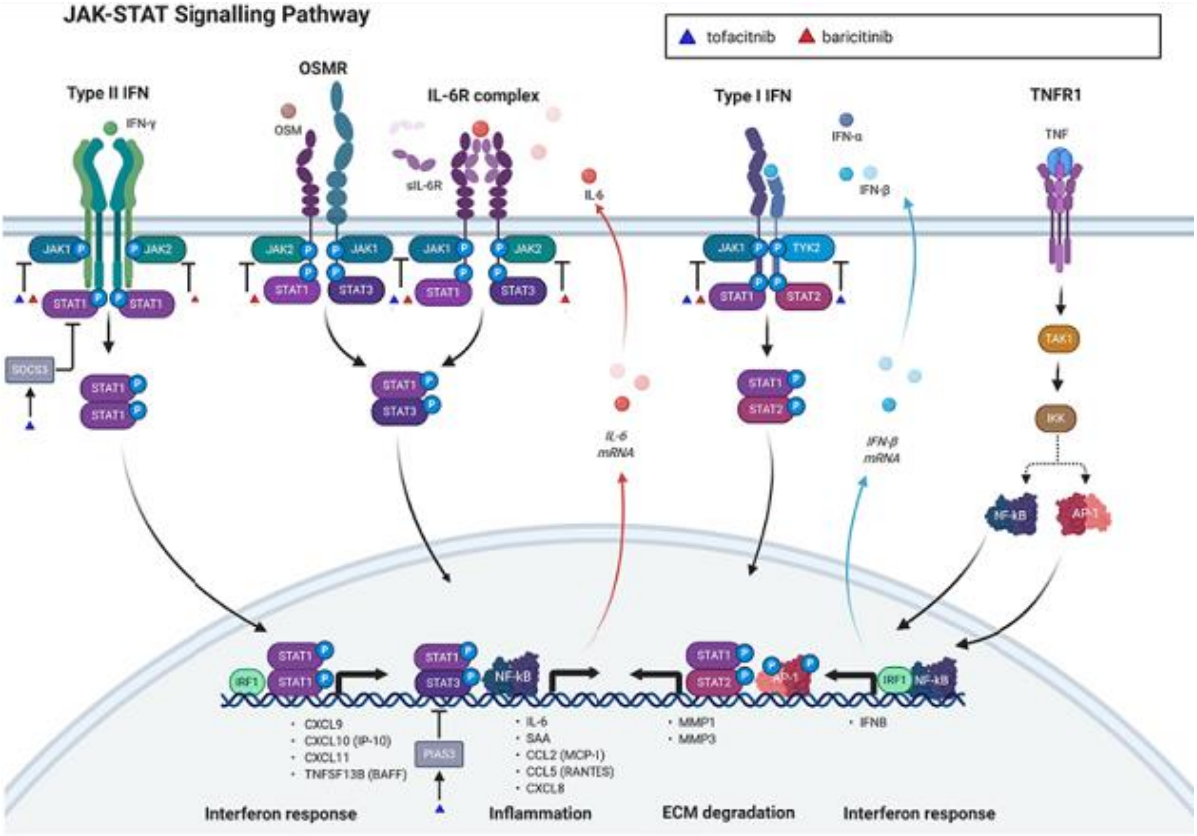


Figure 5 JAK STAT Pathway schematic view of the activation of cytokines and inflammatory gene expression. The figure also illustrates the workings of the JAK inhibitors to downregulate the proinflammatory cytokines. Modified (Burja et al., 2020)

1.7.2 Matrix Metalloproteinases (MMPs) in RA

The MMPs are Zinc (Zn) dependent. They require Zn^{+2} at the active catalytic site to function. These are also endopeptidases. Some other extracellular proteins like collagen, proteoglycans and fiber all are found in the extracellular matrix (Malemud, 2006). These MMPs help in the cleavage of these proteins to make help them in migration from one cell to another and also helping in the tissue remodeling process (Wells et al., 2015). When their functions alter e.g., in cancer when overexploited can lead to metastasis (Shay et al., 2015). MMPs are of various types that includes collagenases, gelatinases, stromelysins, matrilysins, metalloelastase, and membrane type MMPs (Itoh, 2015). MMPs are also involved in chemokine activation. MMP2 and MMP9 are involved in the cleavage of chemokine ligand truncating their structures and

function. On binding of the truncated chemokine ligand to the chemokine receptor, failing to activate the receptor. But if the chemokine is not cleaved and the normal chemokine binds to its receptor will initiate the inflammatory response (Roomi et al., 2017).

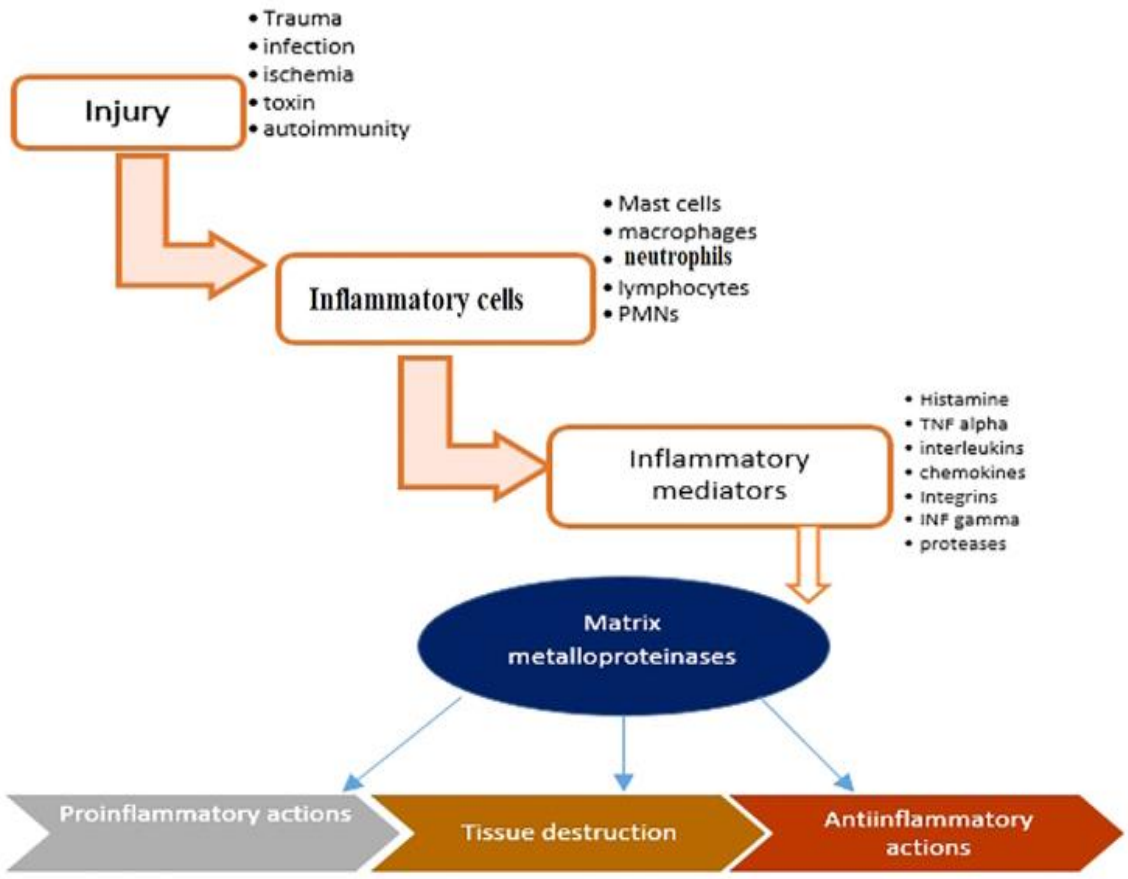


Figure 6 Role of MMPs in Inflammation and anti-inflammation (retrieved from Hasan et al., 2020)

The imbalances of pro-inflammatory cytokines cause the function alteration of MMPs. When the increase in TNF – α occurs, it also increases the synthesis of MMPs which disrupts the levels of chemokines and cytokines (Hasan et al., 2020)

1.7.3 PTGS1 and PTGS2

COX enzymes play an important role in the synthesis of Prostaglandins. COX1 helps in the synthesis of PTGS1 that are essential in the house keeping functions of the

body while the COX2 is needed more in the inflammatory processes. PTGS2 increases the body temperature when causing fever. In order to limit the harmful and painful levels of inflammation the inhibition of COX enzymes can help in the limited production of Prostaglandins (Crofford, 1997). The available commercial drugs; Celecoxib. Lumiracoxib, and Etoricxib selectively target COX enzyme and inhibit them to slow down the progression of the disease (Lipsky & 1997).

Use of medicinal plants to treat various diseases have been highlighted because of the side effects posed by the conventional or synthetic available drugs. Similarly, the drugs of Rheumatoid Arthritis have many side effects and no cure to the disease has been reported yet (Singh et al., 2020).

1.8 Therapeutic Use of Medicinal Plants

1.8.1 Trachyspermum Ammi

Trachyspermum Ammi is a medicinal plant used for various diseases. It belongs from the family ‘Apiaceae’ (Accession No: GBAN- L12673) . It is widely grown and used in South Asia. in Urdu it is also known as ‘Ajwain’. T.ammi is known to have many therapeutic effects, in South Asia it is mainly used for the digestive issues (Mohan et al., 2021). It is known to have anti-inflammatory, anti-hypersensitive and anti-bacterial properties. This medicinal plant have many active components which can be extracted from their seeds using extraction techniques. There is a considerable lack of research and evidence in their anti-inflammatory and anti-oxidant properties. The total alcohol extract (TAE) and the Total Aqueous Extract (TAQ) of T.ammi showed anti-inflammatory effects in animal models (Korani & Jamshidi, 2020). When tested on Arthritic mice models, an increased in the markers related to the anti-oxidant while decrease in the anti-inflammatory markers was observed (Umer et al., 2012)

1.8.2 Colchicum Luteum

Colchicum luteum is another medicinal plant widely used for therapeutic purposes. It belongs from the family, ‘Liliaceae’ (Accession No: 38452). It is mostly found in the Himalayan region and the south Asian countries. There are traces of phenolic compounds found in the extract of the plant (Davoodi et al., 2021). It follows various biological activities e.g., it purifies blood, have anti-cancerous properties, anti-fungal, and anti-inflammatory properties (Reeta et al). It constitutes various active components which also include the alkaloids and therefore also used in arthritic drugs (Ansari et al., 2020).

On conducting the seed extractions using extraction techniques and evaluating the phytochemical analysis it was reported that the alkaloids and phenolic contents were in abundance in C.Luteum (Azabakht., 2020).

2 METHODOLOGY

The methodology includes the series of steps of performing biochemical screening to identify the properties that these plants exhibit based on which identifying the particular bioactive phytochemical that exhibits drug likeness. The shortlisted bioactive chemicals on identifying their potential target proteins can be used in performing the comparative docking analysis using MOE against commercial drugs for RA.

2.1 Phytochemical Analysis

In In-Vitro analysis seeds of C. Luteum and T. Ammi's seeds were collected to make a plant extract. To check the antioxidant activity 2 assays were conducted i.e., DPPH Assay and Albumin Denaturation Assay

2.1.1 Collection and Extraction of Plant

Leaves of the plant T. Ammi and C. Luteum were obtained from the by the help of university, NUST. The leaves were grinded into a refined powder . about 12g of the powder was added in 200 ml of deionized H_2O and was placed in a shaking incubator for overnight at 200rpm. The mixture was then subjected to the to centrifugation for about 20 mins at 6000 rpm and 4ºC temperature.

The mixture was then filtered using the whattsman filter paper of 0.45um pore size. The supernatant was then stored at 4ºC for use in the further assays.

2.1.2 DDPH Assay

DDPH is an anti-oxidant assay which helps in the assessment of the antioxidant activity of the medicinal plants through radical scavenging activity. The results are then evaluated using spectrophotometry.

Dilutions were prepared for both the extracts and the Ascorbic Acid as the control. DPPH solution was made by mixing 5mg DPPH in 100ml methanol. 1ml extract dilutions were added in Eppendorf along with the 0.5ml DPPH. The experiment was run in triplicates for each extract. The dilutions were then incubated at room temperature for 60 mins. The respective OD was then taken at 570 nm.

2.1.3 Albumin Assay

Albumin Denaturation Assay was run to identify the anti-inflammatory activity of the extracts.

Dilution were prepared of both the extracts and ascorbic acid as the control. The reaction mixture was prepared for the assay which contained 0.2ml egg albumin, 2.8ml Phosphate Buffer Saline (PBS) and 2ml extract dilutions. The mixture was then incubated for 15 mins at 37º C. In the next step, the mixture was heated at 70º C and then the ODs were calculated in the UV- spectrophotometer at 660nm.

2.2 In-Silico Analysis

The Gas chromatography-mass spectrometry (GC-MS) data of T. Ammi and C. Luteum was searched and collected from different phytochemical data bases most notably ‘Traditional Chinese Medicine System Pharmacology (TCMSP) database’ (Kim et al., 2015). The other phytochemicals were retrieved from the literature. The

RA Targets

Plant Targets

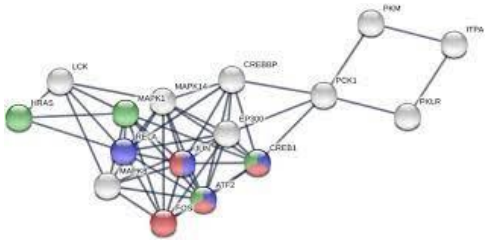
170 targets

9 – bioactive T. Ammi Compounds

9 – Bioactive C. Luteum Compounds

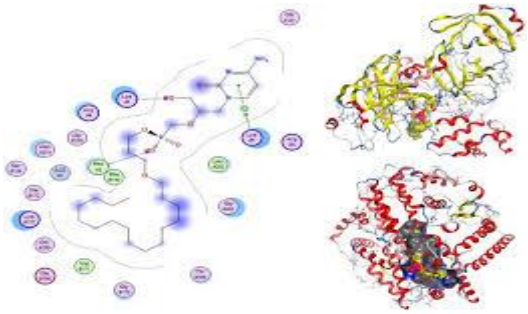
RA Targets → Intersection ← Plant Targets

Cytoscape



Constructed Compound – target – drug Network

Network generated using String App



Comparative Docking Analysis using MOE and analyzed ligand protein Interaction

2.2.1 Phytochemicals Shortlisting

Around more than 1000 phytochemical compounds were collected of both the plants and were shortlisted based on the Lipinski Rule also known as the 'Rule of Five (RO5)' (Pollastri, 2010). This design is initially followed to identify the drug like properties that includes the ≤ 5 hydrogen bond donors which indicates a good absorption property of a compound, ≤ 10 hydrogen bond acceptors, ≤ 500 dalton molecular mass of a compound. This is considered so that the compound is not too large as a standard set for the pharmaceutical property of a drug, Log P that is equal to or less than 5 (Zhang, 2007). Another property that is calculated is the number of rotatble bonds. It is also taken as a selection criteria of the compounds that may exhibit drug like properties. The number of rotatable bonds should be less than 10 which is taken as a good value for the oral bioavailability (Lipinski, 2004). While examining the structure of the compounds of drugs, it was noted that there was not much of the diversity in the compound having more chances of the compound to remain stable (Bemis & Murcko, 1996). These values of the phytochemicals were taken from the Swiss ADME (http://www.swissadme.ch/) that is a web tool having the physiochemical values of components their pharmacodynamic and that helps in examining the drug like properties of each compound based on their canonical smiles (Daina et al., 2017). These canonical smiles were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) which is a database having the information of almost all the chemicals with their structures by their name and formula. It also records all the chemical information of the compounds (Kim et al., 2006). On the basis of these properties, compounds were shortlisted based on their gastrointestinal absorption which was evaluated using the A-Boiled Egg option in SwissADME. The white portion of the egg includes all the compounds that are absorbed in the intestine while the yellow portion includes all the

compounds that pass Blood Brain Barrier (BBB) (Daina & Zoeta, 2016) An illustration of the Egg-Plot is given in the figure 6.

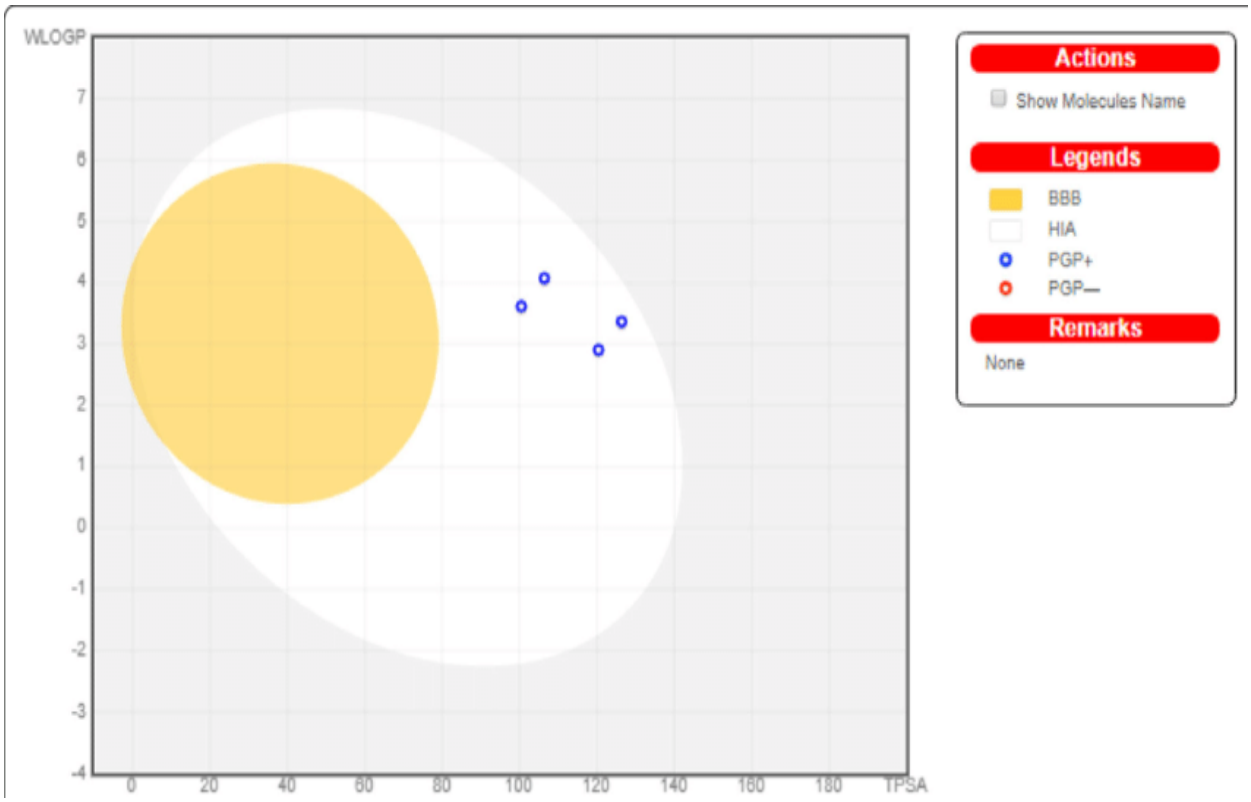


Figure 7 An Illustration of Boiled-Egg generated from SwissADME

Further shortlisting was based on the compounds ADMET properties that includes the Absorption, Distribution, Metabolism, Excretion and Toxicity. These properties of the shortlisted compounds were evaluated using ADMETSar (http://lmmd.ecust.edu.cn/admetsar2/). In this database the Human Oral bioavailability (HOB), Ames Mutagenesis which is the probability of a compound to exhibit a mutation was examined, Carcinogenesis and Acute Toxicity was calculated (Cheng et al., 2012). The compounds were further shortlisted. Based on this 9 active phytochemicals were obtained of both T. Ammi and C. Luteum.

The targets of the plants were retrieved from Swiss Target Prediction (http://www.swisstargetprediction.ch/) which records the data of targets of compounds that are considered as bioactive.

2.2.2 RA Related Targets

Then the targets of Rheumatoid Arthritis were retrieved using the 'Therapeutic Target Database (TTD)', Drug Bank (https://go.drugbank.com/) and literature review was done to collect the data of targets of RA. Around 180 targets were retrieved. These targets were retrieved to identify the common targets of plants and RA (Bai et al., 2021).

2.2.3 Protein-Protein Interaction (PPI) of T.Ammi - RA and C.Luteum - RA

These targets were collected, and the common targets were located of both the plants and the RA. This was done with the help of the String (https://string-db.org/) selecting the option '*Homo Sapiens'*. The String app is used to make the networking locating the protein-protein interaction map as shown in Figure 7. The nodes represent the protein while the edges in the figure represent the interactions of the proteins. The sold lines were made indicating a strong interaction were only considered.

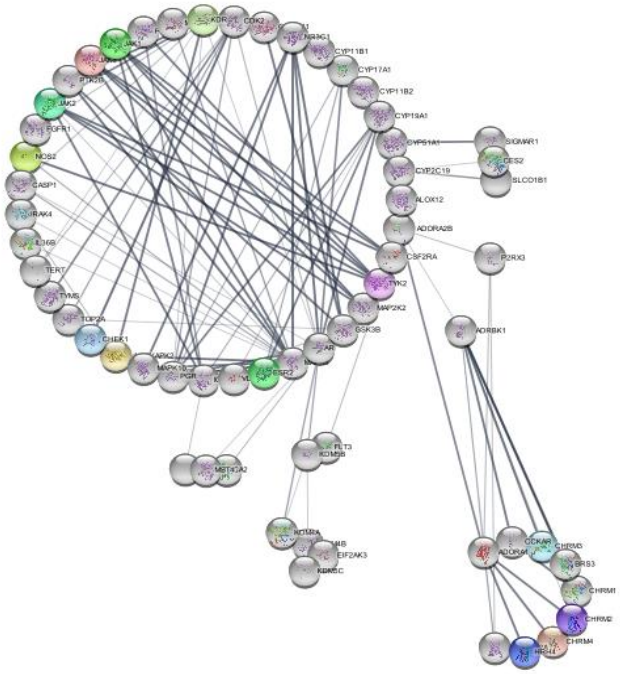


Figure 8 protein network of an active phytochemical of T. Ammi.

This map was exported to Cystoscope tool (https://cytoscape.org/) which is a software used in sorting the complex networks of the proteins and helps in visualizing and analyzing the networking that is formed among the proteins (Saito et al., 2012).

2.2.4 Construction of the Plant – Disease Potential Target Gene Map

The map was generated of each plant with the disease to identify the potential target genes and to analyze the complex networks of the targets. It was done by merging all the networks of each phytochemical by selecting the intersection option as shown in Figure 8 to locate the common potential targets of RA and our desired active phytochemicals (Zuo et al., 2018).

In this networking map, the nodes identify the compound and the target genes when the solid lines indicate the interaction of the proteins and hence the common targets were selected based on this networking.

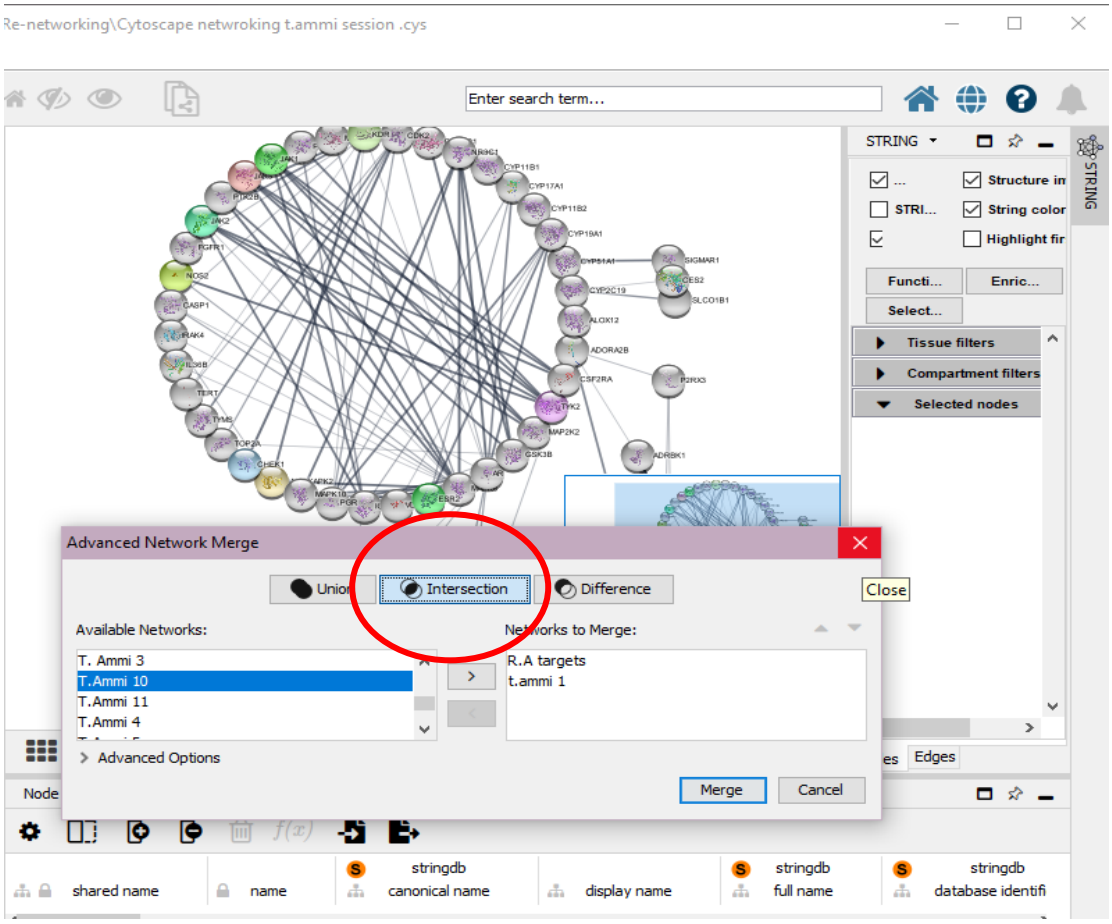


Figure 9 Merging the networks of our desired bioactive targets and the disease targets to generate the common targets by intersection.

2.2.5 Enrichment Analysis

The symbol of the genes were used to generate the Go and KEGG pathways on Enrichr (https://maayanlab.cloud/Enrichr/) that helps to validate the genes involved in the specific pathways (Kuleshov et al., 2016). Their functioning was visualized and analyzed using the p-value that helps in the identification of the probability of the common targets to be involved in the certain pathways. This P-value was considered <0.05 for the analysis.

2.2.6 Comparative Docking Analysis

Docking studies were performed using Molecular Operating Environment (MOE). It is a software that aids in the drug discovery by docking the shortlisted compound to our desired therapeutic target (Vilar et al., 2008). The 3-D structures of

the Target were retrieved form the Protein Data Bank (https://www.rcsb.org/). This database stores the structure of proteins, and ligands. The structures were downloaded in the PDB form. Most of the protein structures had the ligands attached to it which were later removed through the MOE. While the structures of the ligands were retrieved from the PubChem (https://www.uniprot.org/), the structures were downloaded in the .sdf format which were converted into .pdb format. These structures of both the ligands and the targets were uploaded on MOE. A library of ligands were created which docked on the £-D structures of the potential targets. The results were generated in the form of their S-Value and the root mean square deviation (RMSD). The S-value shows the energy needed to make an interaction between the ligand and the target or the drug and the ligand. The lowest S- score indicates the strongest interaction (Attique et al., 2019).

After the interactions, the ligand interaction were evaluated in 2D format and the chemical bonding's were analyzed that made the interaction possible.

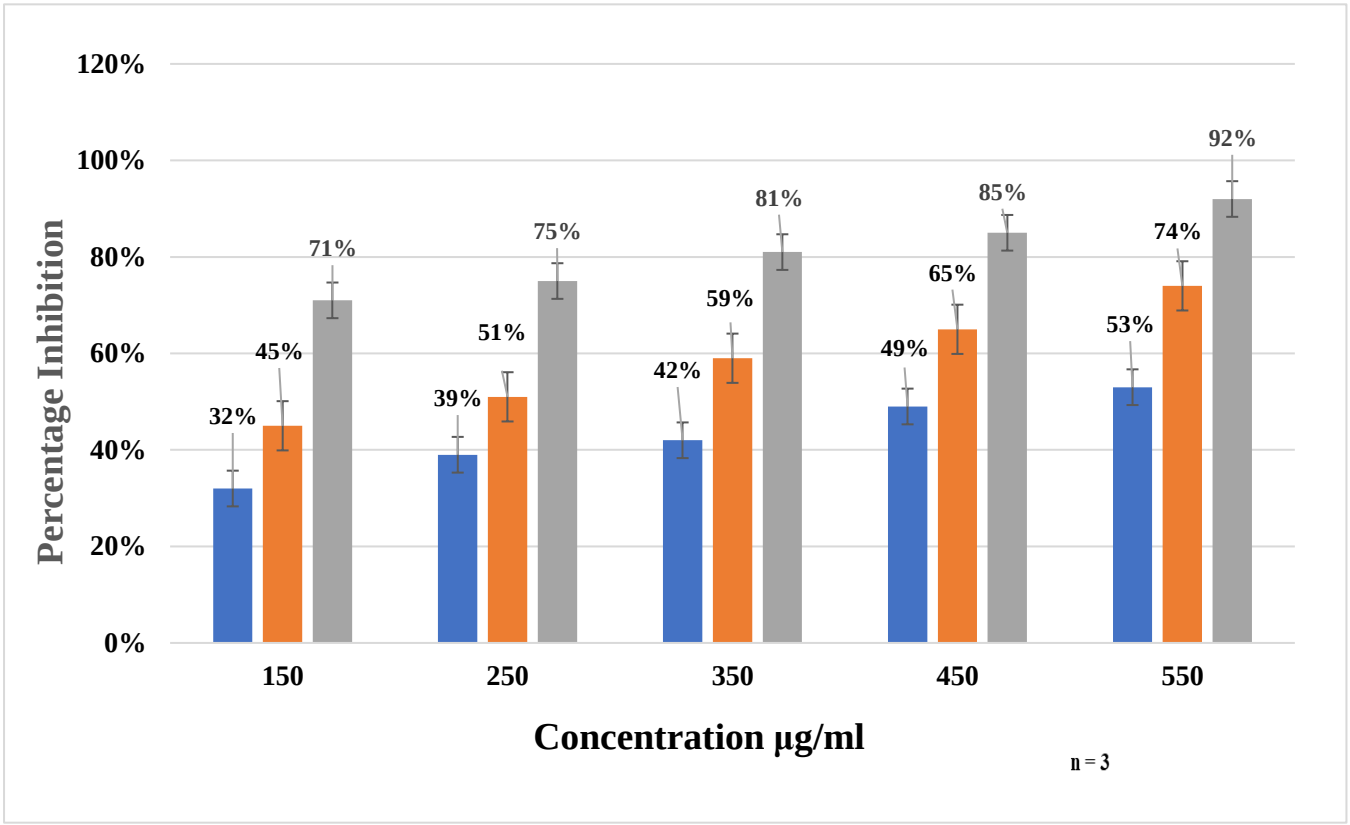
3 RESULTS

3.1 Albumin denaturation Assay

One of the main causes of inflammation reported is the denaturation of proteins. The anti-inflammation property of the denatured proteins is calculated of both the extracts (T. Ammi and C. Luteum).

The inhibition of proteins was calculated using the formula.

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$



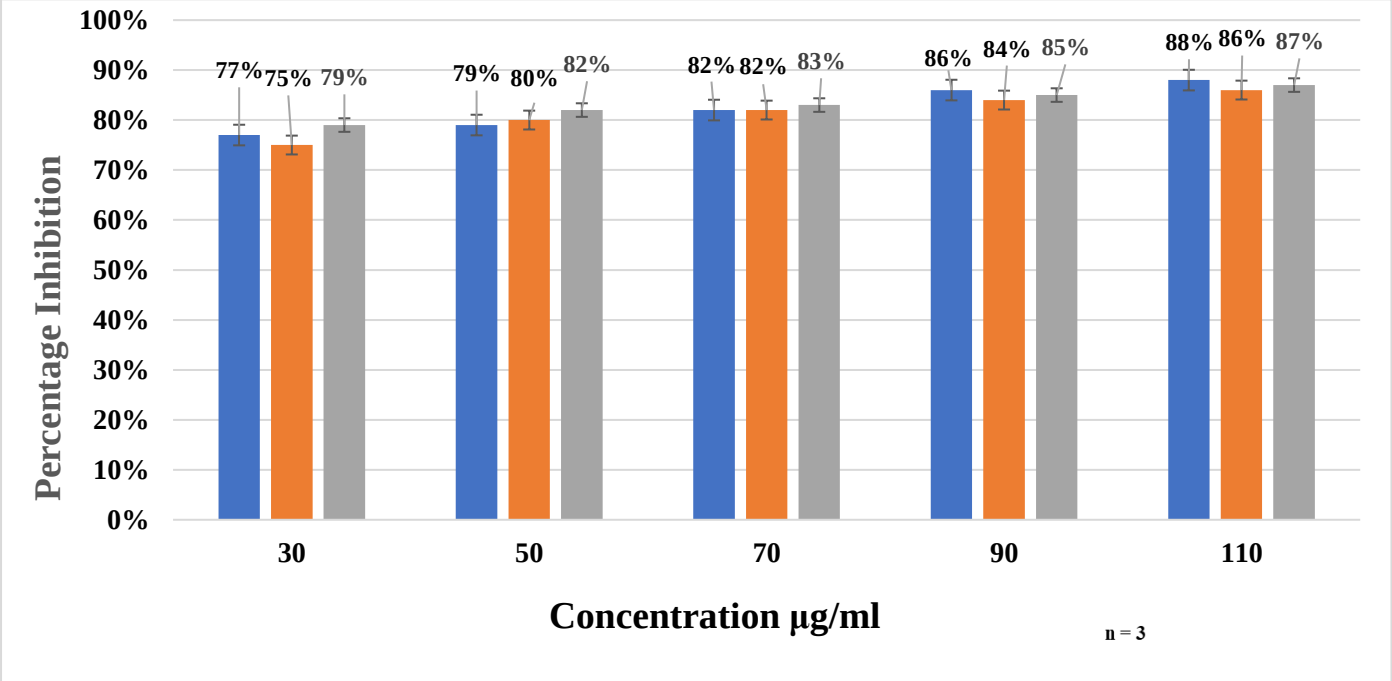
T. Ammi showed higher denaturation inhibition percentage.

3.2 DPPH Assay

The scavenging activity percentage (AA%) was calculated using the formula.

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$

The results showed that all the substances exhibited antioxidant activity, but the antioxidant of T. Ammi is predicted to be higher than that of C. Luteum. The scavenging activity of T. Ammi is more than the C. Luteum according to the results. This assay can be considered reliable and reproducible.



T. Ammi showed higher antioxidant properties as compared to C. luteum

3.3 Compound shortlisting

Following the shortlisting criteria's 9 bioactive compounds were shortlisted of both T. Ammi and C. Luteum.

- T. Ammi Shortlisted Compounds

Compounds	M.WT	H-Donors	H- Acceptors	Rotatable Bonds	Log-P	BBB
4(3H)-Quinazolone, 3-[2-hydroxy-3-(2-oxo-3-propyloxazolidin-4-yl)propyl]-	331.37 g/mol	1	5	6	1.04	No
4-(1H-[1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester	253.26 g/mol	1	5	5	-0.81	No
S-2-[2-Norbornylamino]ethyl thiosulfuric acid	251.37 g/mol	2	4	5	0.86	No
3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane)	420.54 g/mol	0	8	2	-0.66	No
Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester	264.32 g/mol	1	5	4	0.98	No
Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenyl-piperidin-3-yl ester	263.29 g/mol	1	4	3	0.76	No

2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone	346.37 g/mol	3	6	1	1.66	No
1,3-Adamantanediacetamide	281.44 g/mol	3	3	9	0.73	No
Acetamide, N-methyl-N-[4-[4-[2-hydroxyethyl]-1-piperidyl]-2-butynyl]-	250.34 g/mol	2	2	4	1.46	No

- . Luteum shortlisted compounds

Compounds	M.WT	H-Donors	H- Acceptors	Rotatable Bons	Log-P	BBB
Cyclohexanone, 3-carbomethoxy-4-(2'-carbomethoxyvinyl)-4-hydroxy-, TMS derivative	328.43 g/mol	7	6	0	1.85	No
Ethyl iso-allocholate	436.62 g/mol	6	5	3	3.93	No
N-[4'-hydroxy-3-phenylpropionyl]- 5-imidazolethylamine	329.57 g/mol	3	2	0	4.85	No
S-[2-[2-Hydroxy-3-isopropoxypropylamino] ethyl]thiophosphate	259.30 g/mol	7	3	3	1.41	No
Topotecan	273.29 g/mol	9	6	4	0.19	No

1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	421.45 g/mol	3	7	2	1.43	No
2-t-butyl-4,5-bis(ethoxycarbonyl)-1,3,2-Dioxaphospholane	265.35 g/mol	4	2	3	1.68	No
2,4-Benzylidene-d-glucose	266.29 g/mol	3	5	2	1.68	No
3,4-bis(4-hydroxyphenyl)-3,4-hexanediol	342.38 g/mol	3	7	1	1.38	No

3.4 Cytoscape Networking

The network of RA was first generated explaining the interaction among the RA protein as shown in the following figure.

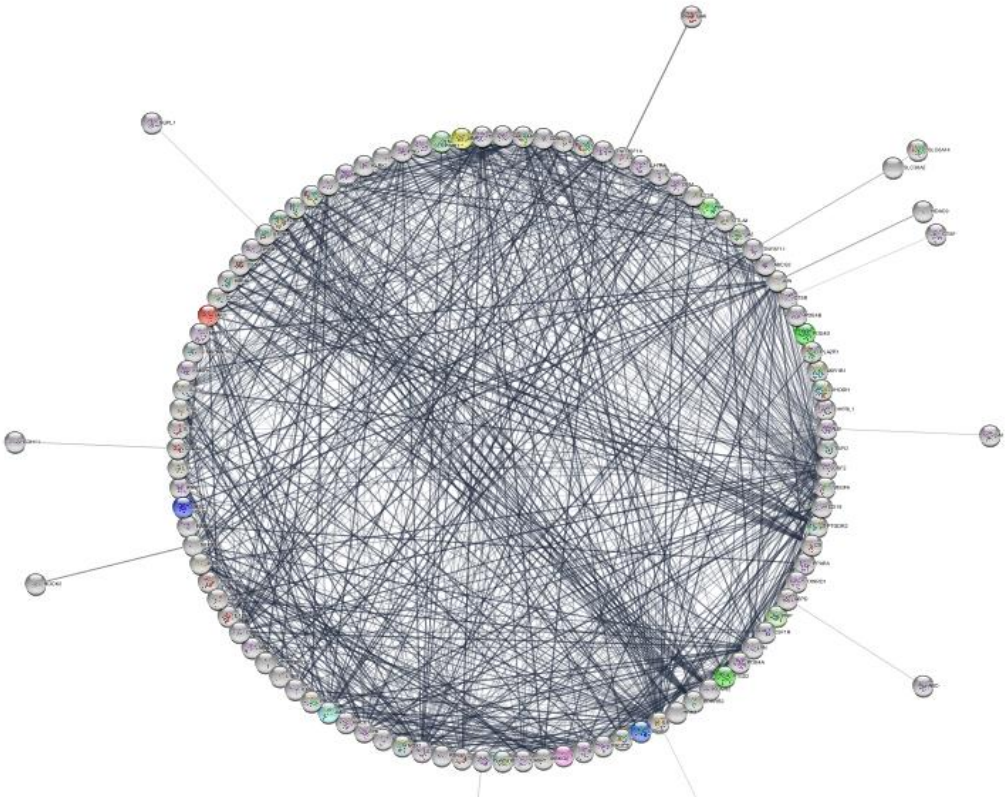


Figure 10 The interaction network of RA proteins

Similarly, the targets of T. Ammi and C. Luteum targets were generated using String and importing the network to Cytoscape. The networks generated of both the plants then were individually merged to retrieve the common targets of RA in both the plants.

- Common Targets of RA and T. Ammi

Target	**Symbols**
Matrix Metallopeptidase 3	MMP3
Prostaglandin-Endoperoxide Synthase 1	PTGS1
Rho-associated protein kinase 2	ROCK2
Protein Kinase C Beta Type	PRKCB
P2X purinoceptor 7	P2RX7
Beta-secretase 1 Precursor	BACE1
C-C Motif Chemokine Receptor 1	CCR1
Prostaglandin-Endoperoxide Synthase 2	PTGS2
Matrix Metallopeptidase 1	MMP1
Janus Kinase 3	JAK3
Janus Kinase 1	JAK1
Carbonic Anhydrase 2	CA2
Aldo-Keto Reductase Family 1 Member B	AKR1B1
Janus Kinase 2	JAK2
Prostaglandin D2 Receptor 2	PTGDR2
C-C chemokine receptor type 5	CCR5
Nitric Oxide Synthase 2	NOS2
Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Beta	IKBKB
Carbonic Anhydrase 1	CA1
Opioid Receptor Delta 1	OPRD1
C-C Motif Chemokine Ligand 2	CCL2
Histamine Receptor H4	HRH4

Tyrosine Protein Kinase	SYK
Bradykinin Receptor B2	BDKRB2
Myeloid cell leukemia-1	MCL1
Bradykinin Receptor B1	BDKRB1
Caspase 1	CASP1
Colony Stimulating Factor 2 Receptor Subunit Alpha	CSF2RA
Phosphodiesterase 4D	PDE4D
Jun Proto-Oncogene	JUN
Nuclear Receptor Subfamily 3 Group C Member 1	NR3C1
Interleukin 1 beta	IL1B
Proteinase-activated receptor 2	F2RL1
purine nucleoside phosphorylase	PNP
Integrin Subunit Beta 1	ITGB1
Histamine Receptor H1	HRH1
Matrix Metallopeptidase 3	MMP3

- Common Targets of RA and C. Luteum

Targets	**Symbols**
Carbonic Anhydrase 2	CA2
Carbonic Anhydrase 1	CA1
Janus Kinase 3	JAK3
Prostaglandin-Endoperoxide Synthase 1	PTGS1
Janus Kinase 1	JAK1
Caspase 1	CASP1
Beta-Secretase 1	BACE1
Janus Kinase 2	JAK2
Tyrosine Protein Kinase	SYK
Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Beta	IKBKB
Prostaglandin-Endoperoxide Synthase 2	PTGS2

Vascular Endothelial Growth Factor A	VEGFA
Matrix Metallopeptidase 3	MMP3
Induced Myeloid Leukemia 1	MCL1
purine nucleoside phosphorylase	PNP
Matrix Metallopeptidase 1	MMP1
Rho-associated Kinases 2	ROCK2
Colony Stimulating Factor 2 Receptor Subunit Alpha	CSF2RA
Bradykinin Receptor B1	BDKRB1
Phosphodiesterase 4D	PDE4D
Aldo-Keto Reductase Family 1 Member B	AKR1B1
Protein Kinase CAMP-Activated Catalytic Subunit Beta	PRKCB
Cannabinoid Receptor 2	CNR2
Purinergic Receptor P2X 7	P2RX7
NADPH Oxidase 1	NOX1
Nuclear Receptor Subfamily 2 Group C Member 1	NR3C1
Protein Tyrosine Kinase 6	PTK6
Interleukin 6 Cytokine Family Signal Transducer	IL6ST
C-X-C Motif Chemokine Receptor 3	CXCR3
C-C Motif Chemokine Receptor 1	CCR1
Opioid Receptor Delta 1	OPRD1
Tyrosinase	TYR

After generating the common targets of bioactive compounds and RA targets, the commercially available drugs were retrieved from the Drug Bank (https://go.drugbank.com/) for the specified targets so that the comparative docking analysis could be performed.

3.5 Docking Results

The active phytochemicals ligand were docked with the desired potential targets and then the Commercial Drugs available were docked on the same target to compare the S-value and rmsd value in order to identify whether the selected phytochemical has high affinity or the commercial drug.

- **Comparative Docking Analysis of C. Luteum and Commercial Drugs**

Target (SYL)	**Phytochemicals of C. Luteum** / **Drug**	**S- Value**	**RMSD**
JAK 1	Ethyl iso-allocholat	-8.01	1.13
	Upadacitinib	-12.3	1.61
JAK 2	3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol	-12.63	10.80
	Barcitinib	-5.25	1.22
JAK 3	Topotecan	-14.24	1.35
	Tofacitinib	-10.3	0.95
PTGS1	3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol	-12.05	1.2
	Dinoprostone	-11.4	1.1
PTGS2	3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol	-12.9334	1.3
	Celecoxib	-8.4	2.0
CASP1	Cyclohexanone, 3-carbomethoxy-4-(2'-carbomethoxyvinyl)-4-hydroxy-, TMS derivative	-7.08	3.26
	Pralnacasan	-6.8	2.1
MMP1	Topotecan	-9.02	2.15
	Regorafenib	-9.5	1.9
	topotecan	-8.9	2

ROCK2	Netarsudil	-8.7	1.63
BDKRB1	Topotecan	-13.4	2
	Captopril	-8.24	1.3
PDE4D	3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol	-11.3	0.9
	Dyphylline	-9.5	2.0
AKR1B1	1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide	-8.7	0.6
	Sulindac	-11.4	1.4
CNR2	1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide	-8.9	0.8
	Lasofoxifene	-11.6	1.3
SYK	Topotecan	-10.5	2.63
	Fostamatinib	-14.7	3.59
PTK6	N-[4'-hydroxy-3-phenylpropionyl]- 5-imidazolethylamine	-8.4	0.7
	Vandetanib	-11.9	2.1

- **Comparative Docking Analysis of T. Ammi and Commercial Drugs**

Targets	**Phytochemicals of T. Ammi**	**S-Value**	**RMSD**
	Drugs		
JAK 1	Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester	-13.364	01.87
	Upadacitinib	-12.15	1.61
JAK 2	3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane)	-17.2966	0.9
	Barcitinib	-5.25	1.22

JAK3	2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone	-16.5164	1.9
	Tofacitinib	-10.3	0.95
PTGS2	4(3H)-Quinazolone, 3-[2-hydroxy-3-(2-oxo-3-propyloxazolidin-4-yl)propyl]-	-9.5	2.2
	Celecoxib	-8.4	2.0
PTGS1	2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone	-10.260	2.05
	Dinoprostone	-11.4	1.1
CASP1	3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane)	-7.407	2.3
	Pralnacasan	-6.8	2.1
SYK	Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl) methyl-, methyl ester	-10.2236	3.05
	Fostamatinib	-14.7	3.59
MMP1	2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone	-9.2291	1.17
	Reorafenib	-9.5	1.9
ROCK2	Acetamide, N-methyl-N-[4-[4-[2-hydroxyethyl]-1-piperidyl]-2-butynyl]-	-8.4708	1.5
	Netarsudil	-8.7	1.63
BDKRB1	3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane)	-12.1	1.3
	Captopril	-8.24	1.3
PDE4D	2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone	-14.1	0.8
	Dyphylline	-9.5	2.0
AKR1B1	3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane)	-20.1	1.3
	Sulindac	-11.4	1.4

This comparative list helps us in the identification of the phytochemicals that can be used for drug repositioning for the specific targets the pathways that helps in the progression of RA.

3.6 Ligand Interaction

The following are the ligand interactions in 2d and 3d of the target and active phytochemical showing the highest affinity value. The structures elaborate the type of bonding in between the ligand and target site. It also shows the list of amino acids that are involved in the interaction.

3.7 C. Luteum and Ligand Interaction

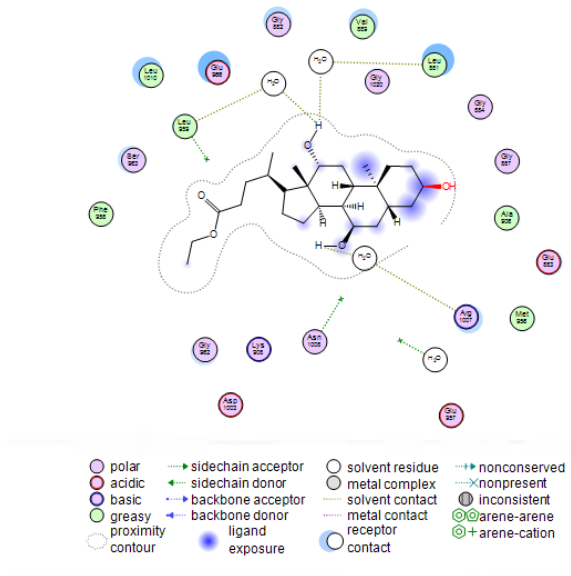


Figure 11 (a) 2D representation of interaction between Ethyl iso-allocholat and JAK1.

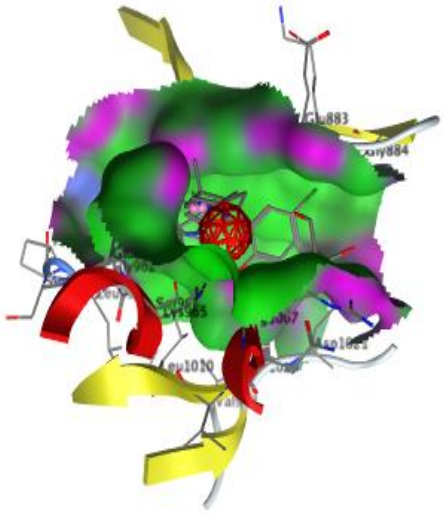


Figure 11 (b) 3D representation of interaction between Ethyl iso-allocholat and JAK1

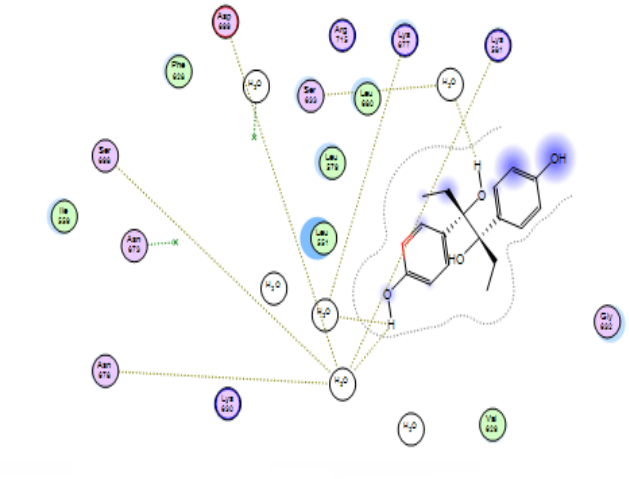


Figure 12 (a) 2D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and JAK2.

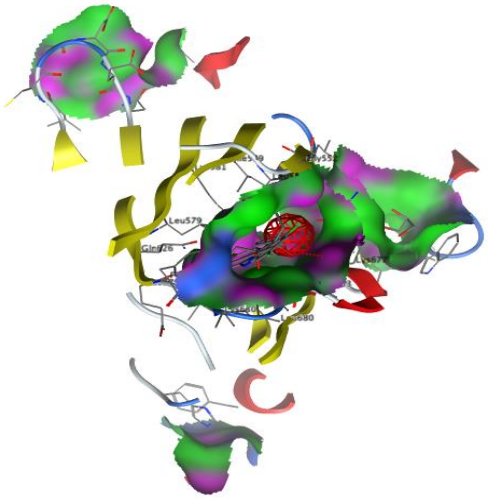


Figure 12 (b) 3D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and JAK2

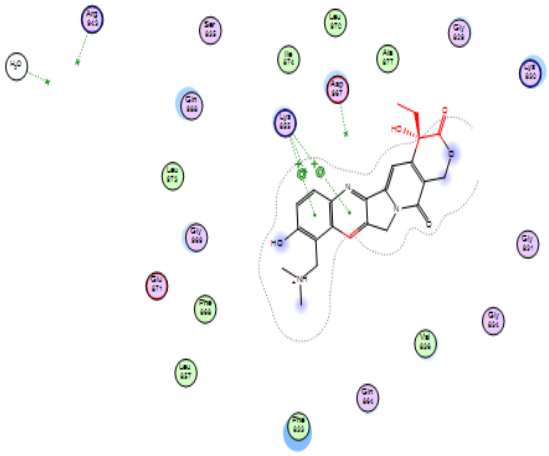


Figure 13 (a) 2D representation of interaction between Topotecan and JAK3

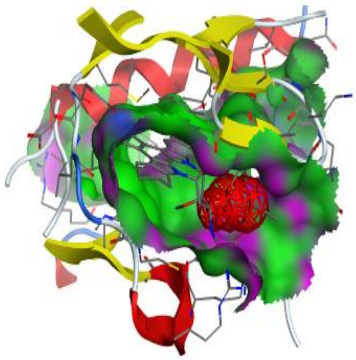


Figure 13 (b) 3D representation of interaction between Topotecan and JAK1

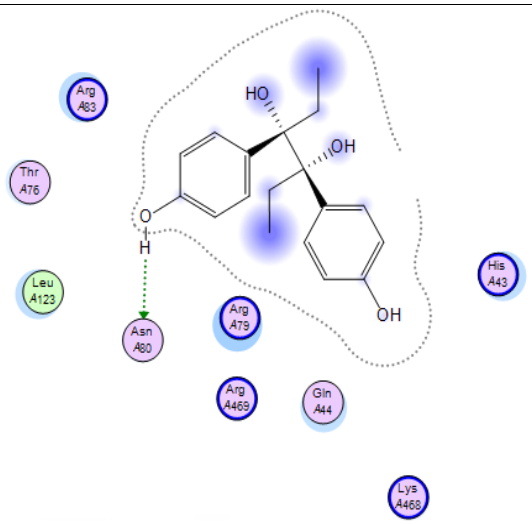


Figure 14 (a) 2D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and PTGS1

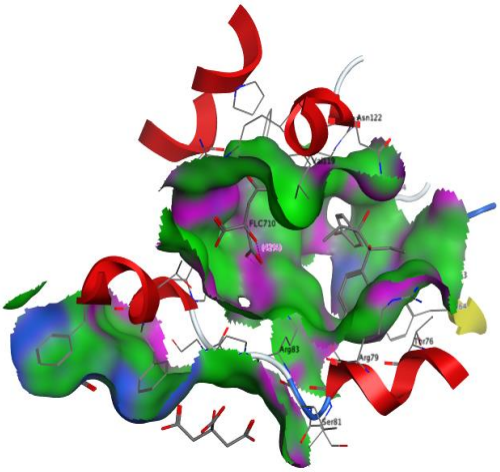


Figure 14 (b) 3D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and PTGS1

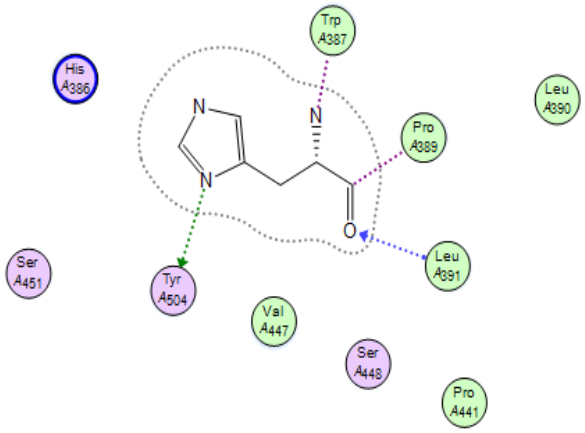


Figure 15 (a) 2D representation of interaction between and 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol PTGS2

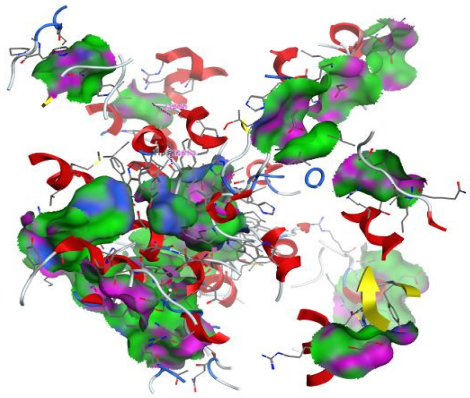


Figure 15 (b) 3D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and PTGS2.

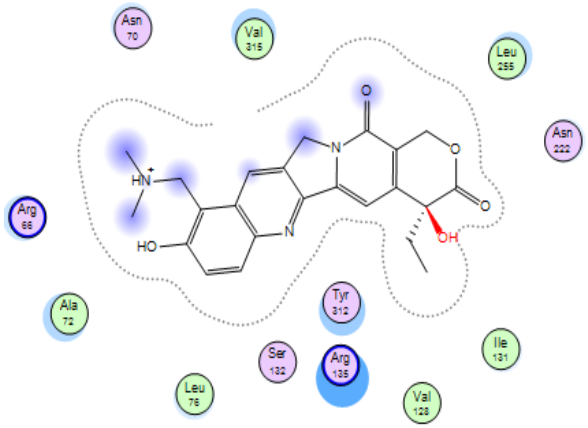


Figure 16 (a) 2D representation of interaction between Topotecan and BDKR1

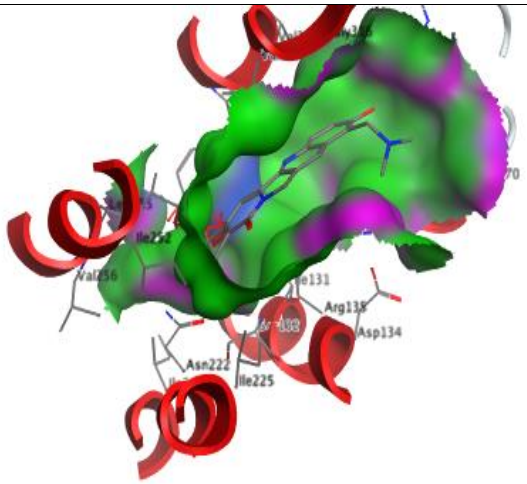


Figure 16 (b) 2D representation of interaction between Topotecan and BDKRB1.

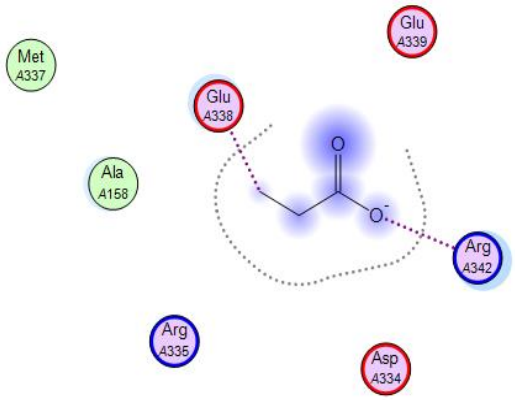


Figure 17 (a) 2D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and PDE4D.

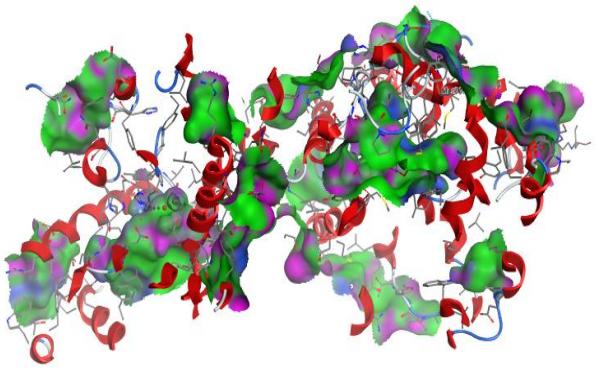


Figure 17 (b) 3D representation of interaction between 3,4-bis(4-hydroxyphenyl)- 3,4-hexanediol and PDE4D.

3.8 T. Ammi Ligand Interaction

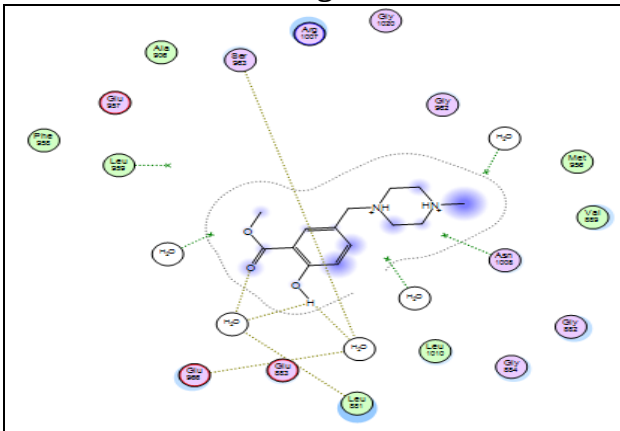


Figure 18(a) 2D representation of interaction between Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl) methyl-, methyl ester and JAK1

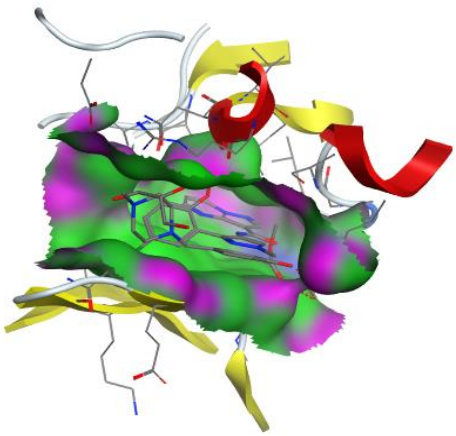


Figure 18(b) 3D representation of interaction between Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl) methyl-, methyl ester and JAK1

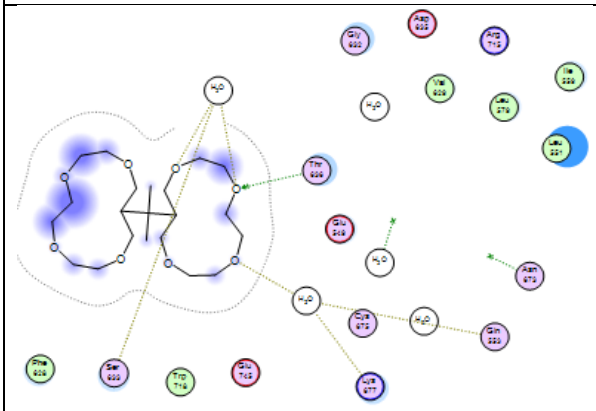


Figure 19(a) 2D representation of interaction between and 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) JAK2.

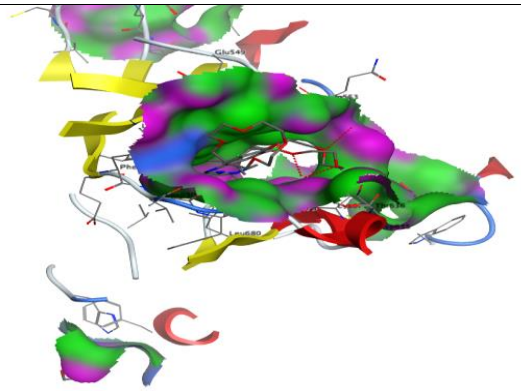


Figure 19(b) 3D representation of interaction between 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) and JAK2.

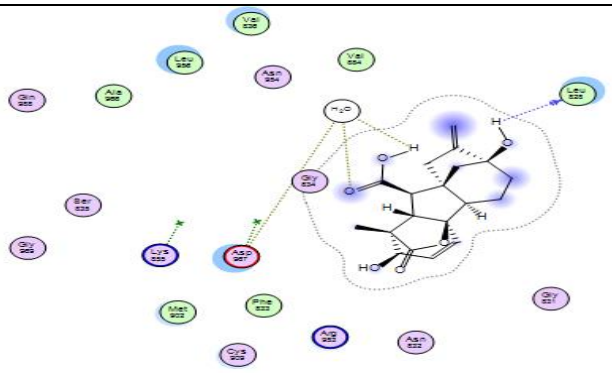


Figure 20(a) 2D representation of interaction between 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone and JAK3.

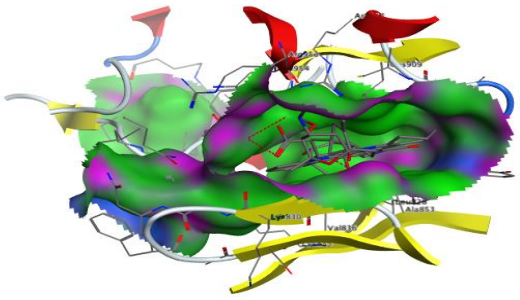


Figure 20(b) 3D representation of interaction between 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone and JAK3.

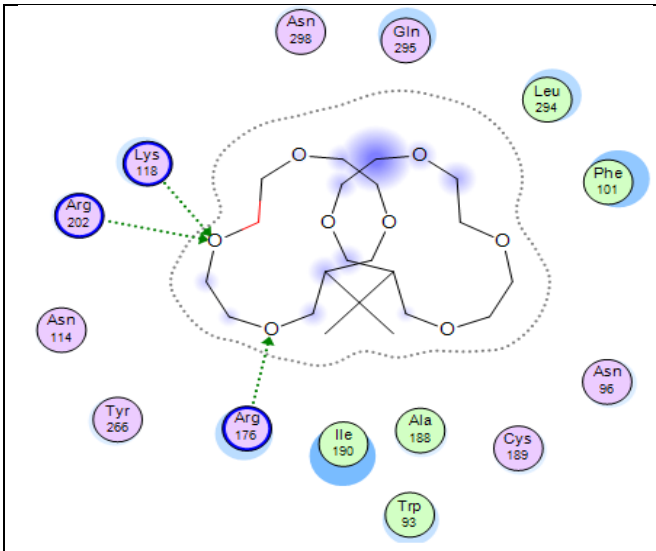


Figure 21(a) 2D representation of interaction between 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) and BDKRB1

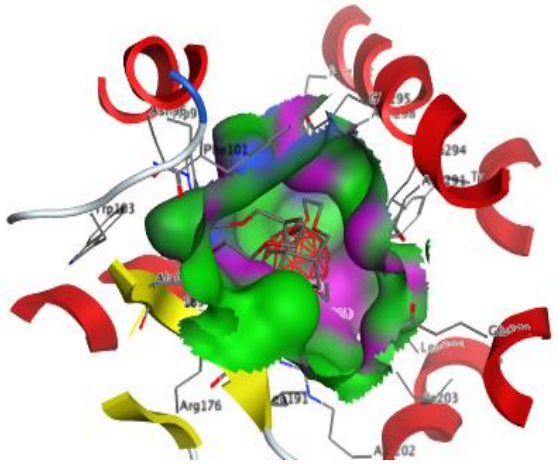


Figure 21(b) 3D representation of interaction between 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) and BDKRB1

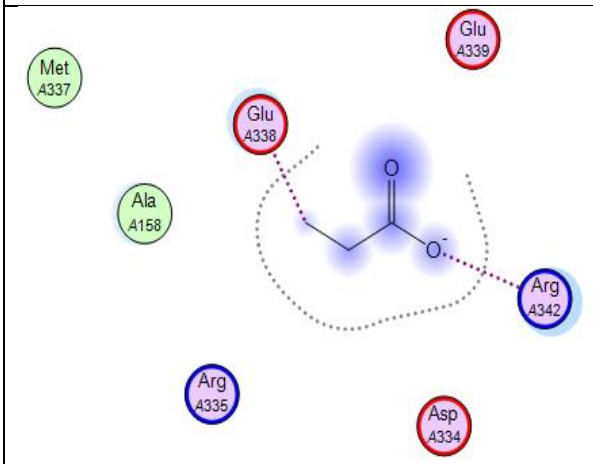


Figure 22(b) 2D representation of interaction between 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone and PDE4D

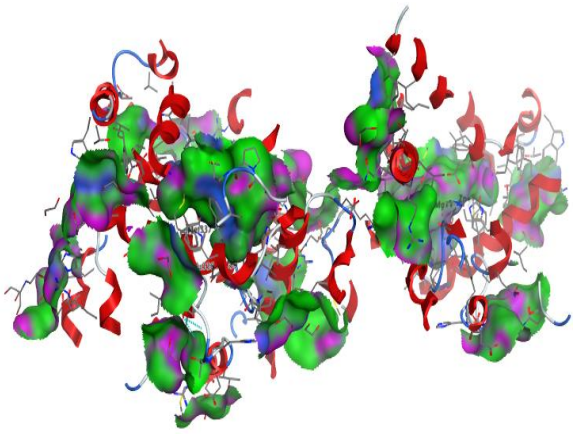


Figure 22(b) 3D representation of interaction between 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone and PDE4D

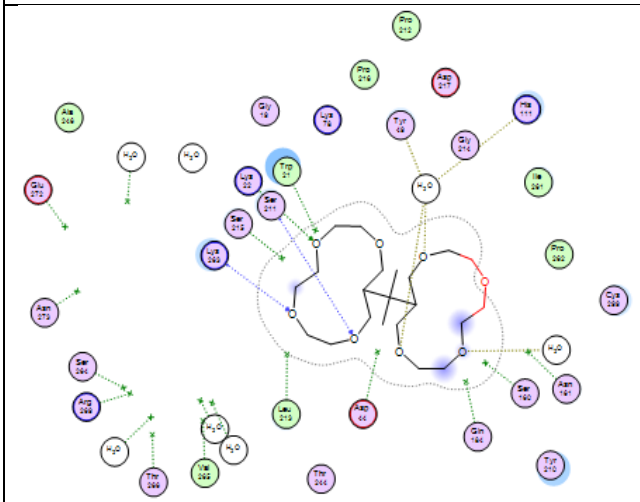


Figure 23(b) 2D representation of interaction between 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) and AKR1B1

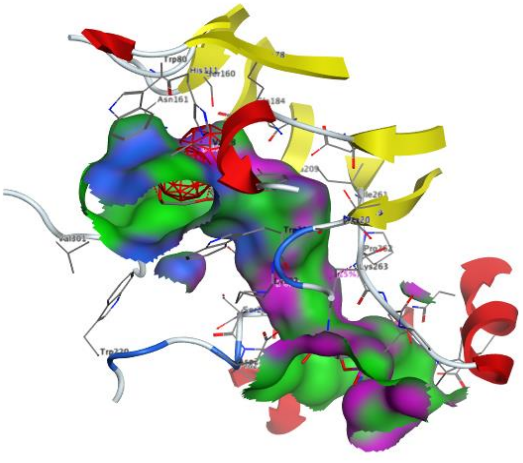


Figure 23(b) 3D representation of interaction between 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) and AKR1B1.

4 DISCUSSION

Many studies are being conducted on *Trachyspermum Ammi and Colchicum Luteum* to check on their therapeutic properties against various diseases. Both plants have shown to exhibit active anti-inflammatory and anti-oxidant activity according the to the existing studies. (Qamar et al., 2020; Ansari et al., 2020) Umar and colleagues (2012) conducted experiment on Collagen induced arthritic mice by administering *Trachyspermum Ammi* extracts. And their study concluded a strong anti-inflammatory and anti-rheumatic activity by limiting and restricting inflammation (Umar et al., 2012). Similarly, Nair and colleagues (2011) conducted study on the properties of *Colchicum Luteum* in arthritic models contributing to the effectiveness of C. Luteum as an anti-inflammatory and anti-granulomatous medicinal drug. It has shown to suppress the inflammation in the affected joints and has also shown to restrict the granuloma formation. C. Luteum has also showed high alkaloid content which validates its effectivity in treating anti-inflammatory diseases (Nair et al. 2011)

This work introduces the significant advantages of medicinal plants and potential target protein in the reduction of RA pathogenicity. Study of medicinal plants have shown that natural compounds, Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester, Topotecon, hexandiol, S-2-[2-Norbornylamino]ethyl thiosulfuric acid) of plant T. ammi and C.luteum showed effective activity with RA targets. These were also under the ADMET toxicity level and lied in Lipinski's rule of 5 and 3. The predicted associations might help in disease inhibition. These compounds can be further studied to evaluate their effectiveness in targeting the potential target proteins involved in Rheumatoid Arthritis as there are no effective medicines available to manage the disease. The comparative docking analysis of the potential drugs and commercialized drugs showed to have a positive results in

inhibiting the pathways that are actively involved in the pathogenesis of RA and have also exhibited the possibility of conducting further studies as an alternative medicine.

5 CONCLUSION

Phytochemicals of *T. ammi* and *C. luteum* from Chinese database TCMS were taken. These phytochemicals were shortlisted through their ADMET properties. Their networking against RA targets were generated and the shortlisted targets were docked with their respective phytochemicals. Docking results showed significant interactions and energy values between RA targets and phytochemicals. There was a significant difference between the energy values of docked phytochemicals versus commercially available drugs. These targets (genes) have significant role in RA pathogenesis. Phytochemicals having significant inhibition role against these targets, have less side effects reported. Whereas the commercial drugs have reported many side effects. In future, these phytochemicals can be investigated in vitro and in vivo against their respective targets to determine their mode of action to reduce RA pathogenicity.

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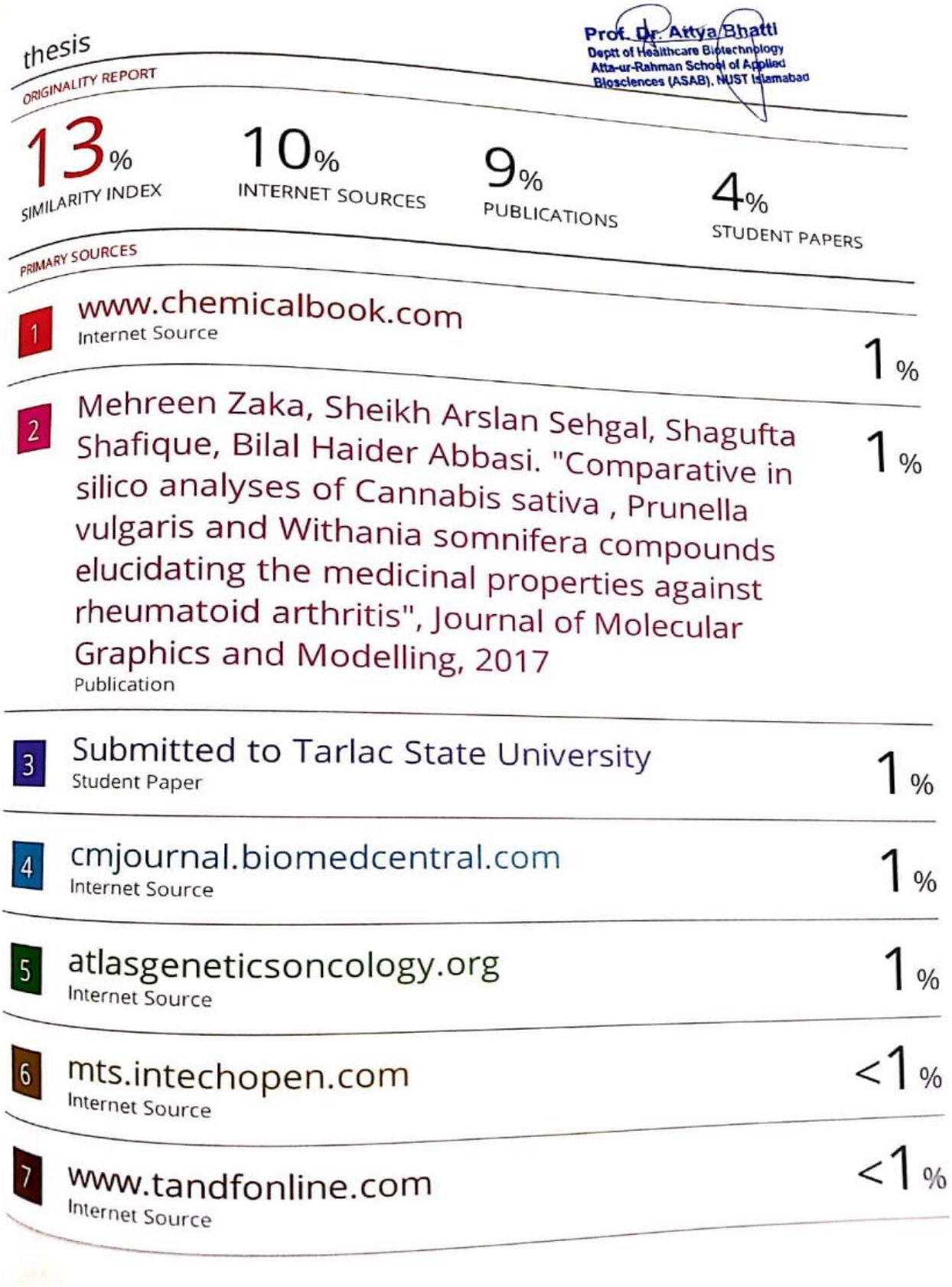

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