# Comparative *In-silico* Evaluation of Therapeutic Potential of *Thymus serpyllum* and *Tachyspermum ammi* against Rheumatoid Arthritis

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# Comparative *In-silico* Evaluation of Therapeutic Potential of *Thymus serpyllum* and *Tachyspermum ammi* against Rheumatoid Arthritis

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# DEDICATED TO MY PARENTS

For the eternal love, support and encouragement throughout this Degree

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## ABSTRACT

Rheumatoid arthritis is an autoimmune, symmetrical polyarthritis characterized by synovial hyperplasia leading to progressive bone and cartilage degeneration. In this study, two medicinal plants namely *Thymus serpyllum* and *Tachyspermum ammi* have been compared for their anti-arthritic potential against rheumatoid arthritis through radical scavenging DPPH assay, albumin denaturation assay and human red blood cell membrane stabilization assay followed by examining their modes of action using in-silico approach. Potentially active compounds from GCMS analysis of methanolic extracts of both plants were screened on the basis of Lipinski rule of 5, ADMET properties and blood brain barrier permeability by utilizing data from online servers like swissADME and admetSAR. Hub gene analysis was done on potential targets of rheumatoid arthritis taken from Therapeutic Target Database (TTD) and two hub genes i.e Tumor Necrosis Factor alpha (TNF α) and Vascular Endothelial Growth Factor A (VEGF-A) were recruited as a result by using String and Cytoscape. All the shortlisted compounds from plant extracts were docked with proteins, TNF-α and VEGF-A and the compounds which exhibited least binding energies with both of the proteins were chosen. Two compounds of *Thymus serpyllum* i.e Erythronolide A, 12-deoxy showed good binding energy with TNF alpha where as 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride exhibited good binding energy with VEGFA. Similarly, two compounds of *Tachyspermum ammi* i.e. Methyl 2-hydroxy-5-[(4-methylpiperazin-1-yl)methyl]benzoate showed least binding energy with TNF alpha and S-2-[2-Norbornylamino]ethyl thiosulfuric acid with that of VEGFA. Results employ that methanolic extracts of both plants exhibited good anti-arthritic potential whereas Thymus serpyllum showed more potent anti-arthritic potential than *Tachyspermum ammi*. In-silico analysis further revealed that compounds of *Thymus serpyllum* showed better binding energies with both hub genes as compared to *Tachyspermum ammi*. In conclusion, *Thymus serpyllum* is a much more potent anti-arthritic medicinal plant than *Tachyspermum ammi* and can be further tested using in-vitro and in-vivo approaches.

# Introduction

## 1.1 Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is an autoimmune arthropathic illness that affects people all over the world and is linked to increasing disability, systemic problems, early mortality, and economical consequences (Firestein 2003). Rheumatoid Arthritis is described as an Inflammatory Arthritis (IA) that fits accepted classification standards, such as the 1987 American College of Rheumatology (Arnett, Edworthy et al. 1988) and the 2010 American College of Rheumatology/European League against Rheumatism criteria (Aletaha and Smolen 2018). It is a long-term disorder characterised by diarthrodial joint inflammation, symmetrical polyarthritis, and synovial hyperplasia (swelling), which leads to progressive bone and cartilage degeneration, loss of articular function, and final joint deformity (Guo, Wang et al. 2018). Pain, weariness, anorexia, stiffness, puffy and sore joints (Mateen, Zafar et al. 2016), as well as a major decline in patient health-related qualities of life, contribute to a large drop in patient health-related attributes of life (Bansback, Marra et al. 2009). Although the exact etiological milieu for RA is unknown, a variety of endogenic and exogenic triggers, as well as genetic predispositions, appear to be associated to increasing autoimmune reactions in the synovial membrane (Edwards, Szczepański et al. 2004). Seropositive RA is defined as an increase in serum levels of autoantibodies known as rheumatoid factor (RF) as well as antibodies to citrullinated protein/peptide antigens (ACPAs). Seronegative RA is defined as having normal serum levels of autoantibodies known as rheumatoid factor (RF) as well as ACPAs (Guo, Wang et al. 2018). The brain (decreased cognitive capacities and

tiredness), lungs (fibrotic problems), exocrine glands (secondary Sjogren's syndrome), liver (increased acute phase response and chronic anaemia), muscles (sarcopenia), and bones (osteoporosis) have all been shown to be affected by RA inflammation. Death rates in affected people are reported to be more than two times as high as in the general masses, and this disparity appears to be widening (Gonzalez, Maradit Kremers et al. 2007).

## 1.2 Rheumatoid Arthritis Epidemiological Features

According to various studies, RA has a global prevalence of 0.5 percent to 1% and is second only to illnesses like osteoarthritis and gout as the leading cause of disability (Naqvi, Hassali et al. 2017). However, research have discovered that the prevalence of RA varies by geographic region and demographic. The prevalence % has been reported in certain studies all around the world. In Serbia, for example, the prevalence is 0.35 percent, with a female predominance (Zlatković-Švenda, Stojanović et al. 2014). A prevalence rate of 0.55 percent has been found in Lithuania, 0.41 percent in Italy, and 0.31 percent in France (Zlatković-Švenda, Stojanović et al. 2014). From the Asian half of the globe, South Korea reported 0.26 percent and Japan between 0.6 percent and 1%. (Dougados, Soubrier et al. 2014). Furthermore, in African countries, a prevalence of 0.9 percent has been found in South Africa, 0.2 percent in Egypt, 0.9 percent in Congo, and 0.13 percent in Algeria, with a RA prevalence rate of less than 0.5 percent in Nigeria (Slimani and Ladjouze-Rezig 2014). Furthermore, in Canada, a disease prevalence of 0.9 percent has been recorded (Ohinmaa, Thanh et al. 2014). India has a frequency of 0.75 percent of RA in South Asian countries (Malaviya, Kapoor et al. 1993). According to a recent survey, female patients have a greater disease rate than male

patients in Pakistan, with a prevalence of 0.142 percent in Karachi and 0.55 percent in northern areas (Alam, Kidwai et al. 2011, Naqvi, Hassali et al. 2017). Incidence and prevalence estimates for RA are two times greater in females than in males, indicating a preference for the female population. Males have a 1.7 percent chance of having RA during their lifetime, while females have a 3.6 percent chance (Crowson, Matteson et al. 2011). The onset can occur at any age, but it is more common in people between the ages of 30 and 50(White and Chang 2008).

### 1.3 Rheumatoid Arthritis Pathophysiology

The synovial fluid and synovial macrophages make up the stratum synoviale of the joints, which is 2–3 cells thick. The stratum synoviale, on the other hand, undergoes rapid hyperplasia and proliferates to a dense thickness of 10–15 cells (Noss and Brenner 2008, Filer 2013). Synovial membrane inflammation is linked to extraneous flooding or native stimulation of mononuclear cells, or both, as well as angiogenesis (Smolen and Steiner 2003), increased expression of adhesive species as a consequence (such as selectins, integrins, and members of the immunoglobulin (Ig) superfamily) (McInnes and Schett 2011). The pannus, or osteoclast-rich area, causes bone destruction, while synoviocytes, neutrophils, and chondrocytes produce enzymes that breakdown cartilage (McInnes and Schett 2011). The cellular composition of normal and affected joints is depicted in Figure 1.1.

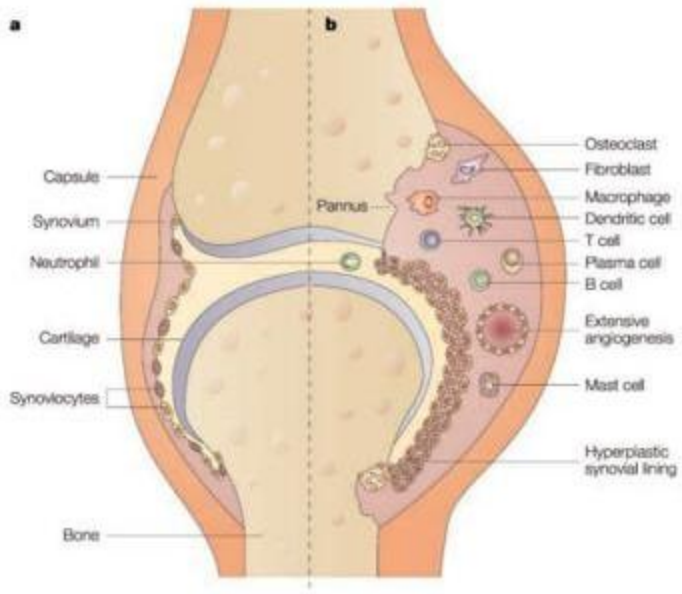


**Figure 1.1. Pictorial depiction of a (a) normal joint and (b) a joint affected by RA (Smolen and Steiner 2003).**

Extra-articular complications (such as rheumatoid lumps, arteritis, uveitis, pericarditis, keratoconjunctivitis sicca, and rheumatic lungs) have also been reported (Hochberg, Johnston et al. 2008), as well as secondary manifestations (such as anaemia, protein synthesis in the acute phase, cardiovascular disorders, osteoporosis, tiredness, and depression) T cells and B cells both play a role in the pathogenesis of RA, with pro-inflammatory cytokines playing a significant role. The activation of the innate immune pathway, which is thought to be the first event in RA, is marked by the stimulation of dendritic cells by exogenous species and autologous proteins (Smolen and Steiner 2003, Smolen, Aletaha et al. 2007). Natural killer cells, neutrophils and mast cells are present in synovial fluid (SF).

Dendritic cells (DCs), a crucial class of antigen-presenting cells that produce a pool of class II HLA molecules, cytokines, and costimulatory molecules near T cell clusters in the synovial membrane, demonstrate the adaptive immune system's significant role in RA. DCs help to stimulate T lymphocytes by presenting antigens to them in the synovial membrane (Lebre, Jongbloed et al. 2008). Following T cell activation, naive T helper (Th) cells split into three distinct subsets (Th17, Th2, and Th1), each with its own cytokine profile and functions (Lohr, Knoechel et al. 2009). Both DCs and macrophages produce interleukin (IL)-1, IL-21, IL-6, IL-23, and transforming growth factor (TGF)-, as well as other cytokines that promote Th17 development while suppressing regulatory T cell production, altering the synovial membrane's homeostatic balance to inflammation (McInnes and Schett 2011). In addition to antigen presentation, B lymphocytes have a role in the pathophysiology of RA by producing cytokines, antibodies, and autoantibodies. Autoantibodies to rheumatoid factor (RF) and anticyclic citrullinated peptides (CCP) are common in RA patients, and they can form huge immune complexes and promote pro-inflammatory cytokines like TNF alpha, by activating complement and Fc-receptors (Smolen, Aletaha et al. 2007). In summary, T and B lymphocyte activation activates a loop mechanism that allows T cells, macrophages, and B cells to interact in new ways (Smolen and Steiner 2003, Smolen, Aletaha et al. 2007). Bone resorption is a clinically frequently reported event in RA patients, and it is caused by monocyte differentiation into bone-resorbing osteoclasts. The cells in arthritic patients' pannus and inflammatory synovium produce cytokines such as IL-6, IL-17, TNF alpha and IL-1 which contribute to inflammation and may have a direct impact on bone health. The OPG

/RANK/ RANKL system, which can be altered by pro-inflammatory cytokines, regulates osteoclastogenesis in the articular erosion of bone in RA.

The discovery of RANKL as a critical regulator of osteoclastogenesis in 1998 opened up new avenues for arthritic-driven bone resorption research. It was later discovered that certain pro-inflammatory cytokines enhance osteoclast development, survival, and function directly or indirectly (Braun, Zwerina et al. 2011).

### 1.4 Reactive Oxygen Species (ROS) and Rheumatoid Arthritis

The significance of reactive nitrogen species (RNS) and reactive oxygen species (ROS) in the pathophysiology of RA is becoming increasingly clear. Both are extremely reactive substances that are likely to damage cartilage cells directly or extracellular matrix mechanisms by boosting matrix degradation mediators. ROS and RNS are produced during normal oxygen metabolism in cells, and when phagocytes are activated, a cascade of unrestrained free radical production damages biomolecules, resulting in altered function and illness. According to the research, ROS damages the activity of the anti-oxidant system, exposing RA patients to oxidative stress and lipid peroxidation as a result of the weakened anti-oxidant defence system, leading to clinical indications of arthritis (Umar, Asif et al. 2012).

### 1.5 Rheumatoid arthritis and Tumor necrosis factor-alpha (TNF-α)

TNF is a key inflammatory trigger in RA, and it promotes osteoclast-mediated bone resorption, it causes considerable damage to joints both locally and systemically. TNF promotes the differentiation of bone-marrow macrophages exposed to RANKL via

increasing the expression of RANKL by B and T lymphocytes, as well as the synthesis of RANKL by osteoblasts (Manara and Sinigaglia 2015). TNF- also inhibits osteoblast proliferation, differentiation, and maturation, which is linked to a reduction in osteocalcin and alkaline phosphatase production (Karmakar, Kay et al. 2010, Manara and Sinigaglia 2015).

## 1.6 Risk Factors leading to Rheumatoid Arthritis

Although precise etiological reasons for RA are unknown, genes, environmental factors, and hormones may have a role in autoimmune development and progression (Iqbal and Rattu 2019). Certain risk factors those are most likely to increase the vulnerability to develop RA, such as old age (those over 60 years old have a higher risk), sex (females have a higher risk), genetics (mostly HLA class II genotypes like HLA-DRB1), a history of live births (mothers who have not given birth before are more susceptible), smoking (cigarettes, tobacco), and obesity (a higher threat with increasing bodily weight) (Aletaha, Neogi et al. 2010). Seropositive RFs and ACPAs affected people have a higher risk. Furthermore, a fascinating study shows that moms who breastfeed their infants are less likely to get RA (Iqbal and Rattu 2019).

### 1.6.1 Genetic Factors

According to numerous research, monozygotic twins have a greater disease concordance of roughly 12- 15% (Lee and Weinblatt 2001, Nakano, Boyle et al. 2013) than dizygotic twins, which has a concordance of 4%, showing that genetic variables play a role in the development of RA. Although there was variability in the degree of disease among twin pairs concordant for RA, heritability analysis in these studies revealed that

genetic variables can explain for 60% of the population's vulnerability to RA (Lee and Weinblatt 2001).

Genetic marker study found an association between RA development and the presence of shared epitope (SE) on small areas of alleles DRB*0401 and DRB*0404, implying that these alleles are linked to chronic disease features such as nodules, rheumatoid factor, erosion, and joint destruction (Lee and Weinblatt 2001, Chabib, Ikawati et al. 2018). The haplotype A1-B8-DR3 is found near the HLA-DPB1 gene, and members of the MHC family, such as PADI4, TRAF1-C5, TNFAIP3, PTPN22, and STAT4, are also linked to a 3-5 percent chance of developing RA (Chabib, Ikawati et al. 2018).

### 1.6.2 DNA methylation

Epigenetics plays a role in pathogenic autoimmunity, as proven by the discovery of an imprinted DNA methylation pattern in RA fibroblast-like synoviocytes (FLS) (Ai, Whitaker et al. 2015). FLS in the intimal lining of the synovium plays a critical role in the development of RA by releasing proteases, cytokines, and small-molecule mediators, while rheumatoid-FLS has an aggressive character that contributes to inflammation and extracellular matrix degradation. Nakano et al. (2013) found that abnormal DNA methylation can affect synoviocyte function and gene expression.

### 1.6.3 Environmental Factors

Hormones, infections, nutritional variables, and cigarette smoke exposure are only a few of the environmental factors linked to the development of RA.

#### 1.6.3.1 Smoking

In 1987, it was first reported that smoking is a key element in the development of RA (Vessey, Villard-Mackintosh et al. 1987). Smoking increases a person's risk of disease by 25%, according to prospective cohort reports and many casecontrol studies, and is thus the biggest factor linked to RA (Costenbader, Feskanich et al. 2006). Multiple studies have indicated that those who have smoked for more than 20 years develop persistent RA. Furthermore, smokers with two copies of the HLA-DRB1-SE allele are more likely to develop RA than persons who do not smoke and have no SE alleles (Klareskog, Stolt et al. 2006).

#### 1.6.3.2 Alcohol

The correlation between RA risk and alcohol intake has been established, and the protective effect of moderate alcohol consumption on the growth of RA has been advised. As a result, researchers have discovered an inverse relationship between alcohol use and the likelihood of developing RA (Hazes, Dijkmans et al. 1990, Maxwell, Gowers et al. 2010).

Several studies have found that alcohol consumption reduces immune response in humans and animals (Fan et al., 2011; Mandrekar, Catalano, Dolganiuc, Kodys, & Szabo, 2004; Verma, Alexander, Carlson, Tygrett, & Waldschmidt, 2008), as well as down-regulate the production of pro-inflammatory cytokines (Waldschmidt, Cook et al. 2008).

#### 1.6.3.3 Reproductive and Hormonal Role

There is a lot of evidence that there is a link between RA and hormones, according to studies. It was found that females are 2 to 4 times more sensitive to disease

than males (Symmons, Barrett et al. 1994). In females, irregular menstrual periods and menarche before the age of ten years may raise the risk of having RA (Karlson and Deane 2012).

#### 1.6.3.4 Infections and Microbiome

A number of scientists are examining the link between viral and bacterial infections and the onset of RA. Experimental investigations have found a link between RA and certain other diseases like the Epstein-Barr virus, gingivitis, and chronic hepatitis C. (Kuwana, Takei et al. 2011, Su, Wu et al. 2014, Kharlamova, Jiang et al. 2016). Furthermore, according to certain findings, a person's microflora can affect the course of RA (Brusca, Abramson et al. 2014, Catrina, Deane et al. 2016, Chen, Wright et al. 2016). Despite the fact that scientists have observed a significant relation between infection, microbiota, and RA, there is insufficient evidence to suggest different causes and effects, necessitating more investigation.

#### 1.6.3.5 Nutritional Intake

There is a relationship between RA and food and nutrient intake, such as vitamin D and protein. Vitamin D has a pleotropic effect on immunological systems, affecting both innate and adaptive immunity pathways via the vitamin D receptor (Arnson, Amital et al. 2007). In collagen-induced murine models, vitamin D has also been shown to prevent the progression of IA (Cantorna, Hayes et al. 1998). Norfolk Arthritis Register (NOAR, Great Britain) discovered an association between red meat protein and the occurrence of RA (Pattison, Symmons et al. 2004).

## 1.7 Diagnosis of Rheumatoid Arthritis

The majority of RA diagnoses are clinical. Polyarthralgia is present, as well as swelling and stiffness at numerous articular sites in a symmetrical, bilateral pattern. Asymmetric oligoarticulation affects certain individuals. Fever, weight loss, fatigue, and/or a lack of strength are all possible side effects (Iqbal and Rattu 2019). Wrists, proximal interphalangeal, metacarpophalangeal, and metatarsophalangeal joints are the most often afflicted joints, whereas distal interphalangeal and spinal joints are generally spared. Reactive arthropathies associated to inflections, spondylarthopathies in seronegative individuals, and other connective tissue illnesses, as well as a variety of endocrine and other ailments, might all be symptoms of RA. During the preliminary diagnostic evaluation, they must be ruled out . In 2010, the American College of Rheumatology and the European League Against Rheumatism teamed up to create table 1.1, an up-to-date diagnostic criteria for RA that aids in diagnosis (Aletaha, Neogi et al. 2010). There is no definitive diagnostic method that can confirm the presence of RA. A full blood profile, differential, rheumatoid factor (RF), and C-reactive protein (CRP) or erythrocyte sedimentation rate should all be included in the initial lab testing (ESR). In certain situations, especially when there are monoarticular presentations, articular suction may be required to rule out viral or crystal-induced arthritis. Furthermore, baseline hepatic and renal function testing are recommended to guide drug selection (Kwoh, Anderson et al. 2002). AACPAs have a better specificity and positive prediction estimate, although they are detected in less than 60% of patients with RA (Van Gaalen, Linn-Rasker et al. 2004). A radiographic examination of the hands and feet should be performed to look for periarticular erosive changes that might suggest a more aggressive RA subgroup (Scott, Wolfe et al. 2010).

**Table 1.1. The 2010 American College of Rheumatology (ACR) classification criteria for RA (Heidari 2011).**

| Category | Explanation | Points |
|---|---|---|
| Joint involvement | >10 joints with at-least 1 small joint | 5 |
| | 1-3 small joints | 2 |
| | 1-3 small joints | 3 |
| | 1 Median-Large joint | 0 |
| | 2-10 Median-Large joints | 1 |
| Duration of symptoms | >6 weeks | 1 |
| | <6 weeks | 0 |
| Acute-phase reactants | Neither ESR nor CRP is positive | 0 |
| | Abnormal ESR or CRP level | 1 |
| Serology | RF or anti-CCP negative | 0 |
| | High positive RF or anti-CCP | 2 |
| | Low positive RF or anti-CCP | 3 |

**1.8**

## Treatment Options for Rheumatoid Arthritis

Nonsteroidal anti-inflammatory medications (NSAIDs), glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and biologics are among the current therapeutic choices for RA patients (Mitragotri and Yoo 2011).

### 1.8.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)

In the early stages of RA, NSAIDs such as celecoxib, indomethacin acetic acid, aspirin, naproxen, and ibuprofen have an analgesic effect. NSAIDs work by inhibiting cyclooxygenases (COX-1 and COX-2), enzymes that generate prostaglandins (PGs) and cause pain and inflammation. However, their use is associated to many problems due to

their ineffectiveness, inability to manage long-term disease development, and numerous side effects such as cardiovascular risk, gastro-intestinal issues, and renal dysfunction (Rao, Knaus et al. 2008, Mitragotri and Yoo 2011).

### 1.8.2 Glucocorticoids (GCs)

Anti-inflammatory mediators such as dexamethasone and prednisolone inhibit phospholipid release, resulting in a reduction in articular inflammation, and can be administered to RA patients during the first two years of therapy. Long-term usage of GCs has been discouraged due to related undesirable consequences such as cardiovascular diseases, osteoporosis, poor glucose metabolism (insulin resistance), skin thinning, hypertension, decreased lesion healing, and obesity. However, about 44 to 75 percent of those who are afflicted take GCs. According to several studies, modest doses of GCs may have disease-modifying benefits in RA patients (Hoes, Jacobs et al. 2010, Mitragotri and Yoo 2011).

### 1.8.3 Disease-Modifying Antirheumatic Drugs (DMARDS)

DMARDs, often known as "slowly acting antirheumatic medicines" (because to the fact that they take 1 to 6 months to take action), are anti-rheumatic pharmaceuticals that were initially used in the late 1980s (von Vollenohower 2009). DMARDs like methotrexate slow down the progression of RA and decrease articular degeneration (McInnes and Schett 2011). Other DMARDs, such as hydroxychloroquine, sulfasalazine, leflunomide, and gold salts, can be used as a replacement for methotrexate or in conjunction with it. Interstitial pneumonitis, myelosuppression, hepatic cirrhosis, retinopathies, hypersensitivity, and allergic responses are also severe side effects of DMARDs (von Vollenohower 2009).

## 1.9 Importance of Herbal Medicines/Phytochemicals

Phytochemicals are active biological agents that are derived directly from plants. They have a wide range of applications in both food and pharmaceuticals due to their outstanding natural characteristics (Georgiev 2014). Phytochemicals are very useful for masking tumours in cancer patients (Singh, Sharma et al. 2016).

Phytochemicals are biologically active constituents of plants that are extracted directly from them. Carbohydrates, polyssacharides, lignins, steroids, flavonoids, stilbenoids, alkaloids, , saponins, and lagnins are only a few examples of phytochemicals that are widely used in pharmaceutical applications and food. (Xiao, Muzashvili et al. 2014), especially in the suppression of tumorigenicity (Singh, Sharma et al. 2016) Furthermore, numerous epidemiological studies have found that consuming phytochemicals on a regular basis is linked to a lower risk of different malignancies (Russo, Spagnuolo et al. 2010).

## 1.10 In-Silico Drug Designing

In silico analysis and bioinformatics have completely transformed the way drugs are designed. It has reduced both the cost and the time spent on medication development. A large number of articles are being produced on medicines and their targets identified using bioinformatics techniques and software. Chemoinformatics advances have broadened the scope of in silico chemical libraries. These compounds may be evaluated for their characteristics and capacity to work as a drug in the human body using contemporary computational techniques. These libraries include both natural and synthetic chemicals, allowing for the customization of a wide range of medicines (Wadood, Ahmed et al. 2013).

### *1.11 Thymus Serpyllum*

*Thymus serpyllum* is a member of the Lamiaceae family, specifically the Thymus family. It is a popular culinary herb. A few species in this genus are used in traditional medicine to treat or prevent illnesses (Organization 1999). The Lamiaceae family is distinguished by thyme essential oil, which contains Phenolic monoterpenes, carvacol, and thymol (Cosentino, Tuberoso et al. 1999). The flavonoids eriocitrin, apigenin, quercetin, and luteolin, as well as rosmarinic acid, make aqueous tea infusions of this family anti-oxidant in nature (Yao, Jiang et al. 2004, Kulišić, Kriško et al. 2007).

*Thymus serpyllum* (wild Thyme) is a herbal plant that is widely utilised for its antibacterial properties and beneficial benefits on the human digestive system. All of the necessary therapeutic qualities are present in this wild thyme (Grieve 2013). Diaphoretic, antispasmodic, sedative, anthelmintic, expectorant, deodorant, tonic, carminative, and disinfection are all properties of the whole plant (Bown 1995, Grieve 2013). Its seeds are also used to treat vermicomposting (Chopra, Nayar et al. 1986). Bronchitis, catarrh, laryngitis, flatulent indigestion, painful menstruation, drunkenness, colic, and hangovers are all treated with it (Bown 1995).

### *1.12 Trachyspermum ammi*

*Trachyspermum ammi* (popularly known as Ajwain), a plant native to Egypt that is also grown in Iran, Iraq, India, and Pakistan, belongs to the Apiaceae family and is very medicinal. *T. ammi* contains carbs, thymol, phenolic compounds, protein, fat, saponins, volatile oil (i.e., para-cymene, -terpinene, and -pipene, ), fibre, and minerals (nicotinic acid, phosphorus, iron, and calcium) according to phytochemical research (Bairwa, Sodha et al. 2012). *T. ammi* possesses various pharmacological properties i.e.,

analgesic, anti-platelet, anti-fungal, bronchodilatory and antitussive, anti-lithiasis and diuretic, hypolopidemic, anti-hypertensive, antiplasmodic (Bairwa, Sodha et al. 2012), anti-bacterial, insecticidal, anti-oxidant, anti-inflammatory, anthelmintic, detoxification, spermicidal, anti-viral, anti-ulcer, digestive stimulant, anti-spasmodic, nematicidal, estrogenic, and hepatoprotective activities (Saleem, Saba Riaz et al. 2017). Thymol, one of the most important components, has anti-inflammatory and antioxidant activities, lowering levels of C-reactive protein (CRP), matrix metalloproteinase 9 (MMP9), IL-6, TNF-, IL-1, and TNF- (Yu, Chao et al. 2016). *T. ammi* has broncho-dilating, antihypertensive, antispasmodic, hepatoprotective (Gilani, Jabeen et al. 2005), anti-aggregant (Srivastava and Acids 1988), antitussive (Boskabady, Jandaghi et al. 2005), anti-aflatoxin (Anilakumar, Saritha et al. 2009).

## Aims And Objectives

- To establish phytochemical libraries of bioactive compounds present in methanolic extracts of *Thymus serpyllum* and *Tachyspermum ammi.*
- Insilico evaluation of these phytochemical libraries with hub genes i.e. VEGFA and TNF, that are active in rheumatoid arthritis.
- To validate results of *in-silico* approaches using in-vitro experiments.

# Review of Literature

## 2.1 Rheumatoid Arthritis

Rheumatoid arthritis is a chronic inflammatory autoimmune disease in which the inflammation does not subside on its own over time and in millions of people, it has become one of the top causes of morbidity.. It is characterised by progressive cartilage erosion as a result of pannus formation, resulting in chronic polyarthritis and joint distortion. In the last decade, advances in molecular biology and early diagnosis have undoubtedly revolutionised therapeutic interventions for improved disease maintenance. Despite the positive outlook, many patients continue to fail to respond to current treatments, necessitating the urgent development of newer, safer medications. Herbal plants have been used since ancient times and have supplied the foundation for a plethora of bioactive substances with therapeutic potential, many of which have been developed into medications that are used worldwide to treat a variety of maladies. In the treatment of rheumatoid arthritis, scientific studies have shown the involvement of several cellular mechanisms such as antioxidant activity , oxidative stress suppression, demoted metalloproteinases induced cartilage destruction, downregulated synthesis of proinflammatory cytokines such as interleukins (IL-1, IL-6), TNF alpha, NF-kB, and increased free radical scavenging. Flavonoids, saponins, terpenes, alkaloids, lactones, and other potent phytoconstituents have been extracted from herbal plants and have been shown to have therapeutic qualities (Santiago, Neto et al. 2021).

Rheumatoid arthritis (RA) is a systemic inflammatory disease marked by inflammation of the synovium that leads to joint erosion and, eventually, deformity. Anti-

inflammatories, immunosuppressants, corticosteroids and synthetic disease-modifying antirheumatic medications (DMARDs) are all used to treat RA. Adverse reactions such as gastrointestinal ulcers, cardiovascular problems, and opportunistic infections are all linked to drug use. As a result, many plant-derived phytochemical substances are being investigated as a potential novel therapeutic method for the treatment of RA. Flavonoids, alkaloids, and saponins are associated to exhibit anti-inflammatory activity and operate as physiological and metabolic regulators among plant phytochemical components for the treatment of RA. Because they are less harmful than other active plant chemicals, their medicinal capabilities are being researched extensively (Santiago, Neto et al. 2021).

## 2.2 Generation of ROS in rheumatoid arthritis

The immune system's oxidation-reduction (Redox) processes are important regulators, and their imbalance contributes to RA progression and maintenance. Oxidative stress (OS) is a condition in which pro-oxidants and anti-oxidants are in an unbalanced state, favouring pro-oxidant events. The oxidative metabolic process produces reactive oxygen species (ROS), which cannot be avoided. (Alcarcaz 2013). The production of reactive oxygen species (ROS) during normal metabolic processes plays a significant role in control of intracellular routes of signalling (Irani 2000). Under normal circumstances, the proper creation of ROS has beneficial effects on a variety of biological processes, including defense from pathogens, tissue repair, and regeneration (Bhattacharyya, Chattopadhyay et al. 2014, Datta, Kundu et al. 2014). Several medicines, smoking, ischemia-reperfusion injury, and inflammatory illnesses, on the other hand, can produce harmful quantities of ROS (Mallick, Yang et al. 2004, Roessner, Kuester et al. 2008, Cederbaum, Lu et al. 2009, Deavall, Martin et al. 2012, Bhattacharyya,

Chattopadhyay et al. 2014). As a result of OS, an unbalanced buildup of ROS causes pathophysiological events such as neurological illnesses, cancer, cardiovascular disease, and chronic inflammation (Halliwell and Cross 1994, Bhattacharyya, Chattopadhyay et al. 2014). As an autoimmune inflammatory condition, RA is accompanied by an oxidative burst that contributes directly to the proliferation and destruction of the synovium (Kundu, Ghosh et al. 2012, Datta, Kundu et al. 2014).

Hypoxia-reperfusion also activates HIF-1 and NF-B, contributing to the persistence of an inflammatory cycle (Alcarcaz 2013). The target genes activated by the hypoxia-induced NF-B signalling cascade are COX-2 and TNF alpha (Schmedtje, Ji et al. 1997, Taylor, Dzus et al. 1998). These processes work together to create a vicious cycle in which the antioxidant defence is overpowered, and the harmful levels of ROS result in OS (Alcarcaz 2013).

The most abundant ROS are O2 and H2O2 (Droge 2002, Margis, Dunand et al. 2008). CAT and GPx scavenge H2O2 (Deisseroth and Dounce 1970, Margis, Dunand et al. 2008). H2O2 is converted to O2 and H2O by CAT, and H2O2 is reduced to H2O by GPx via glutathione (GSH), which is oxidised to glutathione disulfide (GSSG) (Ridgley, Anderson et al. 2018). Lower levels of GSH, an intracellular thiol antioxidant, lead to higher ROS, which causes an imbalance in immunological response, inflammation, and an increased infection risk (Scott, Wolfe et al. 2010). Ridgley et al. (2018) found haem in the reaction centre of CAT and selenoprotein in GSH. The evidence on the effects of antioxidants in RA is contradictory. In RA patients, some antioxidants have reduced levels in both plasma and synovial fluid, whereas others are overexpressed (Alcarcaz 2013). OS-induced impairment in the adjuvant-induced animal model is similar to that

seen in human rheumatic disorders, making it a useful tool for studying OS in autoimmune diseases. The development of an antioxidant to reduce the formation of reactive oxygen species (ROS) has been a fascinating subject of study (Ponist, Zloh et al. 2019).

## 2.3 *Tachyspermum ammi* And Rheumatoid Arthritis

In animal models, total alcoholic extract (TAE) and total aqueous extract (TAQ) of *T. ammi* seeds were found to have a considerable anti-inflammatory activity (Thangam and Dhananjayan 2003). Arthritic mice treated with an aqueous extract of *T. ammi* seeds showed a rise in antioxidant markers and a decrease in inflammation-related markers (Umar, Asif et al. 2012). In a collagen-induced arthritic model, the *T. ammi* extract-treated group had lower COX-2 levels (Korani and Jamshidi 2020).

Varied *Trachyspermum ammi* seed extracts have different anti-inflammatory effects at different dosages. Using n-hexane, chloroform, and methanol as solvents, three distinct seed extracts were generated using the Soxhlet extraction method. Acute toxicity tests at doses starting from 400 mg/kg to 3200 mg/kg were carried out. Wistar rats were administered two different strengths of seed extracts (500 mg/kg and 1000 mg/kg) to test anti-inflammatory efficacy using the Carrageenan induced paw edema technique. When compared to the test drug, the usual medicine diclofenac sodium was more effective (percentage of suppression of paw edema 29.68 percent). When the efficacy of all extracts was examined, the n-hexane extract had the best anti-inflammatory effect (22.21 percent inhibition of paw edema) at the maximal effective dose of 1000 mg/kg. *T. ammi* seed extracts revealed anti-inflammatory action by potentiating GABA neurotransmission and suppressing glutamate receptors (Aslam, Nokhala et al. 2020).

*T. ammi* (L.) seeds are widely utilised in both food and traditional medicine in India and eastern Asia. Fiber (11.9%), saponins, fat (18.1%), carbs (38.6%), tannins, protein (15.4%), glycosides, moisture (8.9%), flavones, and mineral matter are all found in them (7.1 percent ). P-cymene, thymol (50–60%) and -terpinene, and as well as - and -pinenes, -thujen, myrcene, 1,8-cineole, and carvacrol, are all found in essential oil extracted from seeds. Antibacterial, cytotoxic, anti-fungal, anti-lithiasis, anthelmintic, anti-inflammatory, antioxidant, antibacterial, nematicidal, and anti-filarial activity are just a few of the therapeutic properties of ajwain oil. Its seeds are utilised in traditional medicine for their digestive and antiseptic effects. Antispasmodic, antiseptic, antiscorbutic, vermicide, antihistamine, emmenagogue, stimulant, antiparasitic, stomachic, carminative, antihypertensive, aromatic, and sialagogue are some of the other characteristics for them (Anwar, Ahmed et al. 2016).

In mice, *Trachyspermum ammi* essence was utilised to modulate pain scores using the formalin test. A total of 20 male mice (20-25) were used. They were divided into test and control groups at random. Intraperitoneal injections of inhibitors such as naloxone, ondansetron, and atropine were given to the test groups. These specimens were injected with *Trachyspermum ammi* essence after 10 minutes and monitored for 60 minutes to assess the pain effect of formalin injection. the mean pain score throughout the 60-minute formalin test (every 5 minutes) was considerably lower in the groups that got ondansetron, naloxone, and TAE, but the group that received atropine before TAE had a significantly higher pain score (P0.05).The cholinergic signalling may play a role in antinociception. As a result, more research is needed to confirm the essence's impacts on other signalling systems (Borhani, Vahidi et al. 2016).

The antioxidant and free radical scavenging capability of ethanolic extract of *T. ammi* (ESETA) was determined through phytochemical research. For the characterization of key phytochemicals found in ESETA, a preliminary phytochemical screening was performed. ESETA's antioxidant capacity was assessed using DPPH, nitric oxide, superoxide, and hydroxyl radical scavenging models, as well as its ability to prevent lipid peroxidation in bovine brain extract. ESETA's phytochemical investigation confirmed the presence of alkaloids, phenols, glycosides, saponins, terpenoids, and steroids, among other phytoconstituents. The antioxidant capacity was measured by the ESETA as a 73.41 percent suppression of the DPPH radical. The ESETA also demonstrated a 67.33 percent, 63.22 percent, and 62.48 percent inhibitory effect on scavenging nitric oxide, superoxide, and hydroxyl radicals, respectively. Furthermore, in bovine brain extract, the ESETA showed dose-dependent reducing capacity and a notable ferric ion-induced lipid peroxidation inhibitory effect (69.22 percent). *T. ammi's* pharmacological efficacy as a natural antioxidant source has been confirmed by these findings (Bajpai and Agrawal 2015).

## *2.4 Thymus serpyllum*

*Thymus serpyllum* is a member of the Lamiaceae family, specifically the Thymus family. It is regarded as an important herbal plant of ethnobotanical value in several parts of the world, particularly in Pakistan's Gilgit Baltistan region.

### *2.4.1* Pharmacological Properties of *Thymus serpyllum*

Traditionally it is utilized as herbal medicine to treat fever, menstrual issues, gastrointestinal problems, Eczema, Rheumatism, Sedative, Wounds, cholesterol, swellings, flu, bronchitis, diarrhea, respiratory issues and liver and kidney issues.

In nature, there are several chemotypes of *Thymus serpyllum* L. medicinal plant, depending on the geographical region, climatic circumstances, and growing environment. The core groups of biologically active chemicals can differ in composition and quantitative content, resulting in a wide range of biological capabilities (Ivasenko, Orazbayeva et al. 2021).

The essential oils of aromatic plant species of the genus Thymus, popularly known as thyme oil, have a wide range of therapeutic qualities, including antirheumatic, antiseptic, antispasmodic, antibacterial. The oil also aids in the prevention of colds, flu, infectious disorders, and chills by increasing the immune system. It has been shown to be a urinary antiseptic, and it can aid with cystitis and urethritis. Traditional usage of *Thymus serpyllum* essential oils were scientifically validated, and phytochemical and bioactivity evaluations of essential oils from *Thymus serpyllum* were carried out. *T. serpyllum* oil had substantial antibacterial activity against all strains. In addition, *T. serpyllum* oil showed excellent antioxidant activity in all tests and was much effective against all cell lines examined, with GI50 values ranging from 7.02 to 52.69 g/mL. *T. serpyllum* also exhibited no toxicity in swine liver primary cell culture at the studied concentrations (>400 g/mL). The enormous potential of tested Thymus essential oil for using in oral disorders and anticancer treatments, in addition to their common traditional use in food and cosmetics, encourages further exploration (Nikolić, Glamočlija et al. 2014).

Wild thyme *(Thymus serpyllum L.)* is a perennial shrub found to northern and central Europe. Because of its pharmacological qualities, such as antioxidative, antibacterial, and anticancerogenic effects, wild thyme essential oil is increasingly used

in modern medicine. The essential oil's antioxidative and antibacterial activities are linked to the synergistic and cumulative action of its constituents. Further research into the effects of essential oil is needed in terms of antitumor and cytotoxic activity, with the goal of enhancing its cytotoxic effects so that appropriate drugs can be formulated. (Jarić, Mitrović et al. 2015).

Many different active compounds are present in *T. serpyllum* that makes it so much important medically. Different extracts of *T. serpyllum* have been made and tested in different as shown in table 2.1. Whereas different phytochemicals are extracted and also tested for different pharmacological properties as shown in table 2.2.

**Table 2.1. Ethno-pharmacological properties of different extracts of *T. Serpyllum***

| ***T. serpyllum* Extract** | **Pharmacological Activity** | **References** |
|---|---|---|
| Ethanol | Anti-oxidant | (Joshi and Juyal 2018) |
| | Anti-bacterial | (Abramovic, Abram et al. 2018) |
| | Anti-diabetic | (Mushtaq, Bashir et al. 2016) |
| Aqueous-Ethanol | Anti-tumor | (Aralbaeva, Mamataeva et al. 2017) |
| | Cytotoxicity | |
| | Hepato-protective | |
| | Lipid Peroxidation | |
| Aqueous | Anti-hypertensive | (Mihailovic-Stanojevic, Belščak-Cvitanović et al. 2013) |
| | Anti-diabetic | (Mushtaq 2017) |
| | | (Mushtaq, Bashir et al. 2016) |

| | | |
|---|---|---|
| | Anti-inflammatory | (Algieri, Rodriguez-Nogales et al. 2014) |
| | Anti-microbial | (Ultee, Bennik et al. 2002) |
| Methanol | Epigenetic modifications | (Bozkurt, Atmaca et al. 2012) |
| | Anti-cancer | |
| | Cytotoxicity | |
| Hexane | Anti-tumor | (Baig, Ahma et al. 2014) |
| Ether | Anti-diabetic | (Mushtaq, Bashir et al. 2016) |

**Table 2.2. Phytochemicals from *T. serpyllum* and their pharmacological properties**

| **Phytochemical** | **Pharmacological Property** | **Reference** |
|---|---|---|
| Thymol | Antiseptic | (Nikolić, Glamočlija et al. 2014) |
| | Anti-microbial | |
| | Anti-proliferative | |
| | Anti-cancerous | |
| p-cymene | Anti-bacterial | (Ultee, Bennik et al. 2002) |
| Carvacrol | Anti-bacterial | (Jaafari, Mouse et al. 2007) |
| | Anti-cancerous | |

# Materials and Methods

## 3.1 *In-silico* Identification of Lead Compound

### 3.1.1 Drug-Like Properties

Gas-Chromatography Mass Spectrophotometry (GCMS) results of methanolic extracts of *Thymus serpyllum* and *Tachyspermum ammi* showed that there are 763 compounds present within *Thymus serpyllum* extract and 937 compounds present within *Tachyspermum ammi* extract. The purpose of this study was to identify the lead phytochemicals with minimum side effects, which could be used as a future anti-arthritic drug, figure 3.1 represents the whole strategy that was adopted for this purpose. In the first step of in-silico work, drug-like properties of all compounds were assessed. SwissADME software (Daina, Michielin et al. 2017) was adopted for this purpose. SwissADME assesses the drug-like properties of a compound on the basis of five different rules namely; Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule (Bathen and Linder 2017). Each of these mentioned rules evaluate drug-like properties on different parameters which are shown in table 3.1. Compounds violating any one or more of these rules were eliminated whereas rests of them were further evaluated for ADMET properties i.e. Absorption, Distribution, Metabolism, Excretion and Toxicity.

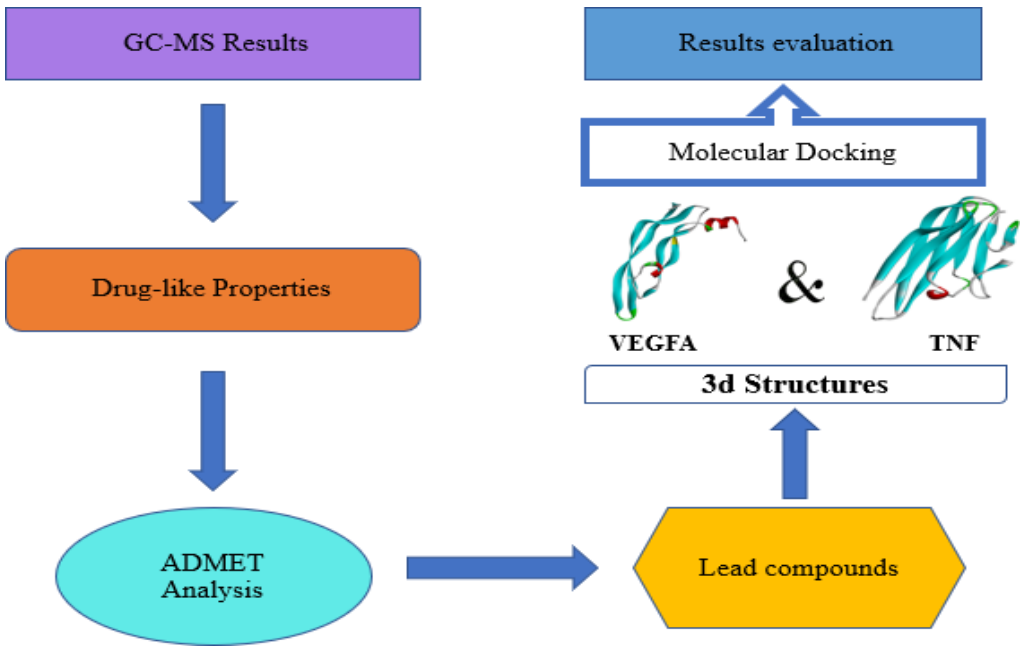


**Figure 3.1 Schematic diagram of the strategy that was used to identify lead compounds for the treatment of Rheumatoid Arthritis**

Blood Brain Barrier (BBB) and Human Intestinal Absorption (HIA) were calculated by Brain Or IntestinaL EstimateD (BOILED- Egg) permeation method which was developed through SwissADME software. BOILED EGG plot examine the polarity [Topological Polar Surface Area (TPSA)] and lipophilicity (WLOGP) of compound and predict its pharmacokinetics with respect to BBB and HIA (Daina and Zoete 2016). TPSA is explained as the aggregate of surface of polar atoms present in the compound chiefly nitrogen, oxygen and attached hydrogen. Compounds having TPSA of more than 140 angstroms squared are poor in absorbance in GI tract while TPSA of lesser than 80 is needed to cross the BBB (Pajouhesh and Lenz 2005). Lipophilicity (abbreviated as logP) is defined as a molecular factor that codes for both electrostatic and hydrophobic intramolecular and intermolecular forces of interactions. Compounds that are highly hydrophobic in nature gets partitioned within the lipid portion of cell membrane therefore

can’t penetrate through the BBB, therefore, LogP value in the range of 0.5 to 5.9 can cross the BBB but the compounds lies in the range of 1.5 and 2.7 are the best ones to cross BBB (Hansch and Leo 1979). Figure 3.2 shows the BOILED EGG plot (taken from SwissADME), if the point of a certain compound comes in the white portion of BOILED EGG then it has high probability to get absorbed within the GI tract. Points located in yellow portion (depicted as yolk of the egg) have the high probability of crossing the BBB and get contact with CNS. There is a grey area in the graph as well; compounds which are predicted to be unabsorbed through GI tract or BBB are pointed in this grey area.

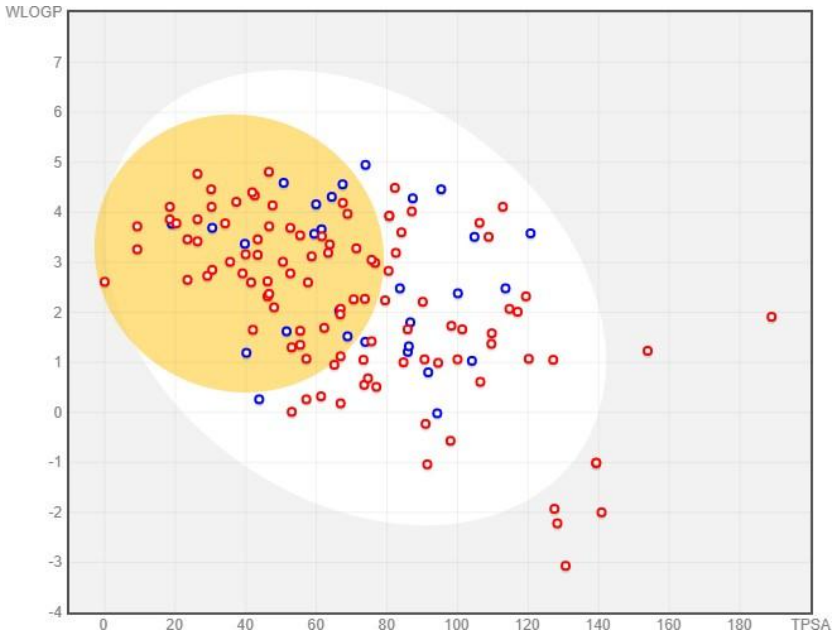


**Figure 3.2. SwissADME generated BOILED EGG PLOT of *Tachyspermum ammi* compounds, where yellow zone is yolk of the egg ( having compounds which can cross the BBB whilst white region is egg white ( containing compounds which can be absorbed through GIT). X-axis represents the Topological Polar Surface Area whereas Y-axis shows Lipophilicity.**

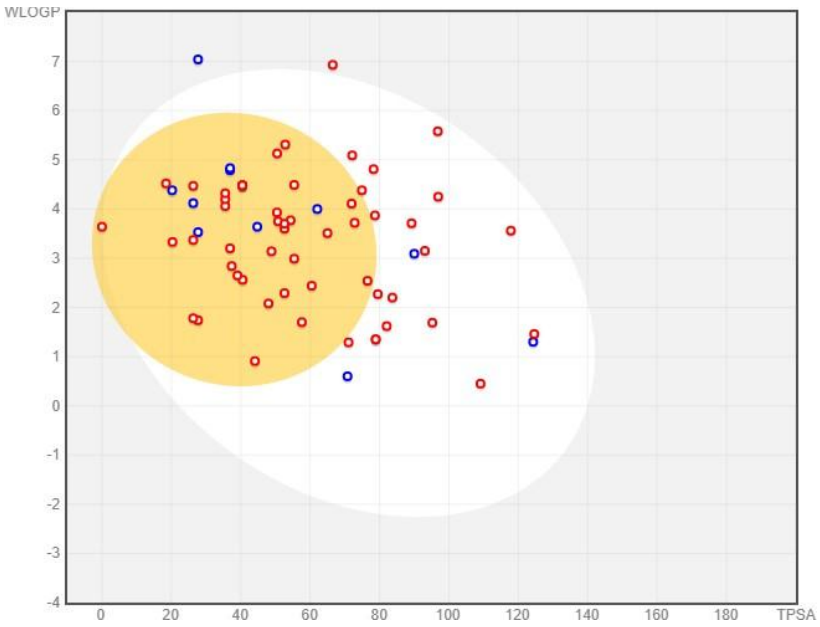


**Figure 3.3. SwissADME generated BOILED EGG PLOT of *Thymus serpyllum* compounds, where yellow zone is yolk of the egg ( having compounds which can cross the BBB whilst white region is egg white ( containing compounds which can be absorbed through GIT). X-axis represents the Topological Polar Surface Area whereas Y-axis shows Lipophilicity**.

ADMET analysis is important to assess the behavior, toxicity and fate of a drug candidate. It predicts the ability of a candidate to cross the blood brain barrier, how well is it absorbed in intestine, its metabolism within the body and further distribution at subcellular level (Lin, Sahakian et al. 2003). In order to estimate the ADMET characteristics of short listed candidates, online tools ADMETsar (Yang, Lou et al. 2019)

and SwissADME (Daina, Michielin et al. 2017) was adopted (Yang, Lou et al. 2019) The analyzed factors of ADMETSAR are shown in table 3.2.

**Table 3.1 Factors and their ranges, on which different rules of drug-likeliness evaluate a compound**

| Rule | Parameters | Range | Reference |
|---|---|---|---|
| Lipinski rule of five | Molecular weight | <500 Da | (Lipinski, Lombardo et al. 1997) |
| | Hydrogen bond Donors | < 5 | |
| | Hydrogen Bond Acceptor | <10 | |
| | lipophilicity of ClogP | <5 | |
| Ghose filter | ClogP | -0.4 to 5.6 | (Ghose, Viswanadhan et al. 1999) |
| | molecular weight | 160 to 480 | |
| | molar refractivity | 40- 130 | |
| | total number of atoms | 20- 70 | |
| Muegge rule | molecular weight | 200 to 600, | (Muegge 2003) |
| | XLOGP | -2 to 5 | |
| | Total Polar Surface Area | ≤ 150 | |
| | Number of rings | ≤ 7 | |
| | Number carbon | > 4 | |
| | Number heteroatoms | > 1 | |
| | Num. rotatable bonds | ≤ 15 | |
| | Hydrogen bond Acceptor | ≤ 10 | |
| | Hydrogen bond Donors | ≤ 5 | |
| Egan rule | WLOGP | ≤ 5.88 | (Tabachnick, Fidell et al. 2007) |
| | Total Polar Surface Area | ≤ 131.6 | |
| Veber rule | Rotatable bonds | ≤ 10 | (Veber, Johnson et al. 2002) |
| | Total Polar Surface Area | ≤ 140 | |

**Table 3.1: Parameters of pharmacokinetics that are assessed by ADMETsar online Server**

| ADMET Property | Analyzed Parameter |
|---|---|
| Adsorption | Caco-2 Permeability |
| Toxicity | Human Ether-a-go-go-Related Gene Inhibition |
| | AMES mutagenesis |
| | hepatotoxicity |

### 3.1.2 HUB Gene Analysis

#### 3.1.2.1 Target Hunting

The targets of rheumatoid arthritis were recruited from Therapeutic Target Database (TTD). Gene symbols and their uniprot IDs were taken from Uniprot. 131 rheumatoid arthritis targets were taken from TTD.

#### 3.1.2.2 HUB Gene Recruiting

All the genes of rheumatoid arthritis were uploaded on STRING software for making a network between these genes that are regulated in rheumatoid arthritis, from where they were exported to Cytoscape. Cytoscape has an extension known as cytoHubba which was used for extracting the most common hub genes of rheumatoid arthritis by applying different criteria including, MCC, EPC, MNC, DEGREE and eccentricity.

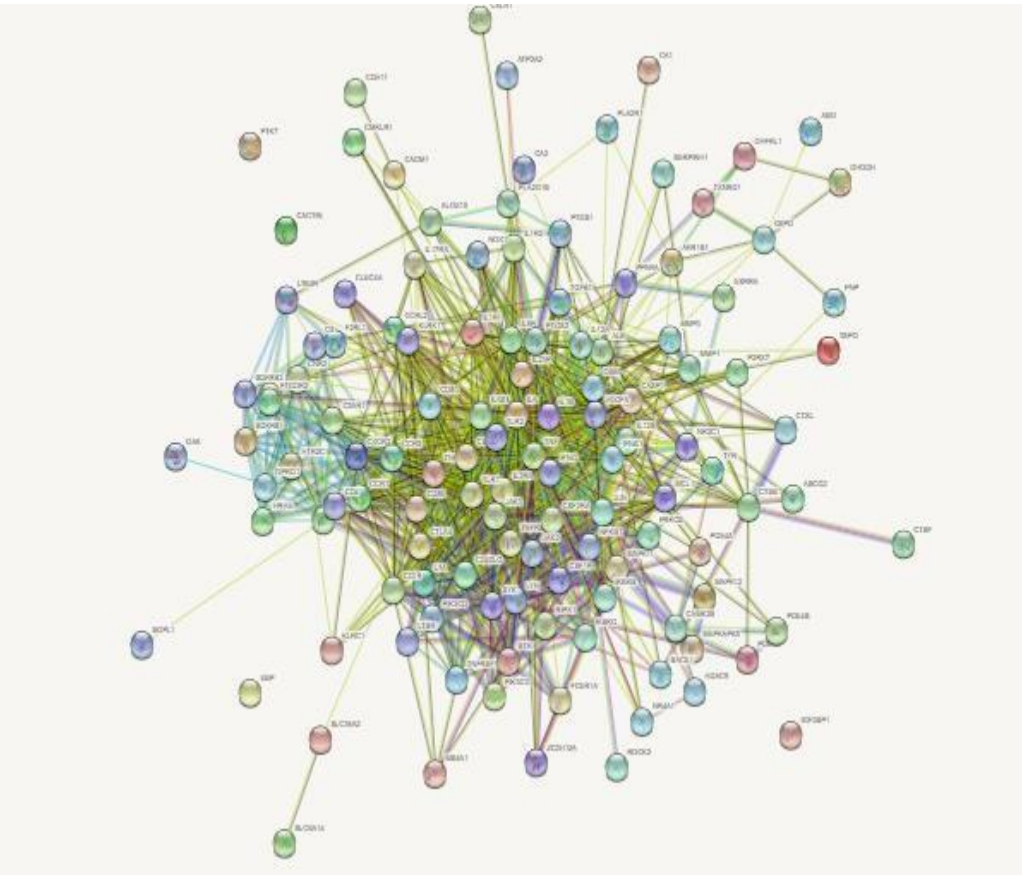

**Figure 3.4 Gene network of rheumatoid arthritis formulated through STRING**

## 3.2 Docking Analysis

### 3.2.1 Establishment of Compounds Library

Once compounds are shortlisted on the basis of drug-like properties and pharmacokinetics, their three dimensional structures were downloaded from Pubchem database (Kim, Thiessen et al. 2016). 3D structures of these entire prospective drugs like compounds were opened in a single library by using Molecular Operating Environment (MOE) software. To make these structures ligand for docking analysis energy

minimization was done via AMBER99 force-field, followed by protonation via Protonate3D algorithm (Labute 2007).

### 3.2.2 Preparation of Target Protein:

For docking analysis TNF alpha and VEGFA were taken as hub genes of arthritis. 3D structures of these proteins were available on Protein Data Bank (PDB) (Rose, Bi et al. 2012) therefore they were downloaded as PDB ID. The protein structure preparation includes protonation via Protonate3D algorithm and AMBER99 force-field was applied for energy minimization.

### 3.2.3 Molecular Docking

3D structures of TNF and VEGFA were then docked with libraries containing 3D structures of phytochemicals of both plants by means of "Molecular Operating Environment" (MOE) software (Roy, Luck et al. 2007).

## 3.3 In-Vitro Analysis of Plants' Extracts

### 3.3.1 Preparation of Extract

Extracts were prepared by dissolving 50mg of grounded plant in 500 ml of ethanol and distilled water separately, the ratio being 1:10. The mixtures were then kept in a dark chamber and agitated thrice a day for ten days. The solutions were ten filtered using a filter paper and funnel. The filtrate was kept in chiller for further use. Some of the filtrate was then air dried to achieve concentrated extracts of *Tachyspermum ammi* and *Thymus serpyllum* (Haider, Li et al. 2009).

### 3.3.2 Phytochemical Testing on Extracts

#### 3.3.2.1 Alkaloids

The test performed for alkaloids is known as Hager's test. 2 ml of extract are taken in a falcon and few drops of hager's reagent are added to it. Formation of yellow precipitates indicates the presence of alkaloids.

#### 3.3.2.2 Phenols

The reaction mixture for phenol testing includes 1 ml of extract and few drops of $FeCl_3$. Appearance of bluish black coloration shows presence of phenols.

#### 3.3.2.3 Tannins

The test performed for tannins is called Braymer's test. To 2 ml of extract, 2 ml of distilled water and few drops of $FeCl_3$ (5%) were added. Greenish to black coloration indicates the presence of tannins.

#### 3.3.2.4 Terpenoids

Reaction mixture contains 2 ml extract, EtOH, 2 ml $CHCl_3$ and 3 drops of concentrated $H_2SO_4$. Appearance of deep red coloration indicates the presence of terpenoids.

#### 3.3.2.5 Flavonoids

The reaction mixture for flavonoids test contain 1 ml of extract and 1 ml of lead acetate. Yellow precipitate formation indicates the presence of flavonoids.

#### 3.3.2.6 Anthocyanins

2 ml of extract was taken in a falcon tube. 2 ml of HCl was added to the extract after which 1 ml of $NH_3$ was poured and color change of pinkish red to bluish violet indicates the presence of anthocyanins.

### 3.3.3 DPPH Assay

To find out the antioxidant potential of methanolic extracts of *Thymus serpyllum* and *Tachyspermum ammi*, DPPH assay was conducted. The purpose of this assay is to identify the capability of analyzed compounds to hunt the antioxidants towards it. The procedure described in previous study (Sanganna, Chitme et al. 2016) was adopted with very few alterations. Following steps were involved in DPPH assay

1. The powdered methanolic extracts of both plants were diluted to varying concentrations of 20- 100µg.
2. Ascorbic acid was taken as control therefore same concentrations of ascorbic acid were prepared.
3. DPPH solution was diluted with methanol such that the final concentration was 2:50. The final solution was placed in a dark room and to provide low temperature it was place on ice.
4. 1ml of each concentration of control and1 ml of each extracts' dilutions were taken in Eppendorf and 0.5 ml of DPPH solution was mixed in them.
5. Step 4 was followed by 1 hour incubation period at room temperature (in dark).
6. Optical Density (OD) was calculated at 517 nm. While taking OD blanking step was done by consuming solvent, which was used in preparation of concentrations of powdered extracts and control i.e. methanol.

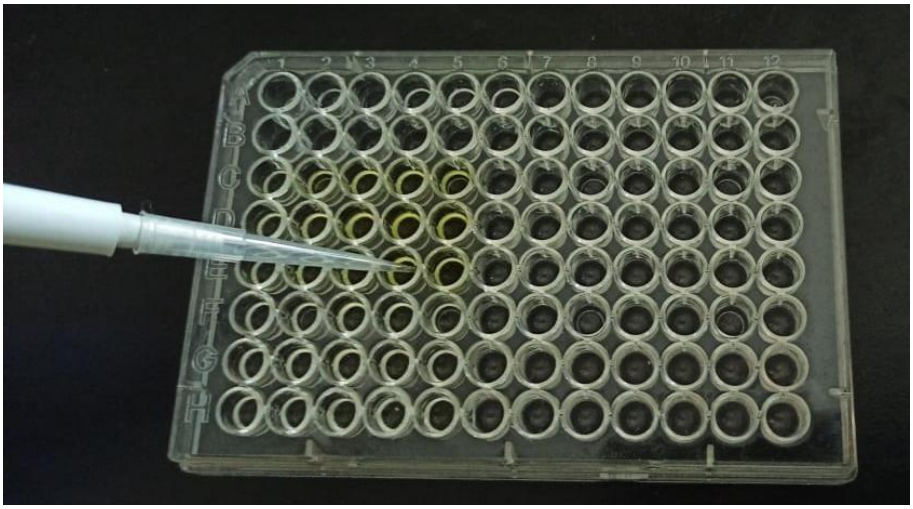

**Figure 3.5 DPPH reaction mixtures being prepared in 96 well microplate**

### 3.3.4 Albumin denaturation assay

Albumin denaturation assay was adopted from (Chandra, Chatterjee et al. 2012)with slight modifications.

1. Different dilutions (200, 400, 600, 800 and 1000 µg/mL) were prepared of dried methanolic extracts of Thymus serpyllum and Tachyspermum ammi and ascorbic acid which was used as a control.
2. To make the reaction mixture, combine 2 mL test dilution, 2.8 mL phosphate buffer solution (pH 6.4), and 0.2 mL egg albumin.
3. Reaction mixtures were incubated for 15 minutes at 37 $\pm$ 2 $^{0}$C.
4. 4. The reaction mixtures were heated at 70°C for 5 minutes after incubation.
5. After cooling, absorbance of the reaction mixtures were measured at 660 nm.

percentage inhibition of protein denaturation was calculated using the following formula:

% age inhibition = 100 * (Vt / Vc - 1)

Where, Vt = absorbance of test sample,

Vc = absorbance of control.

### 3.3.5 HRBC Membrane Stabilization Assay

The assay protocol was adopted from (Shailesh, Seema et al. 2011) with slight modifications in it. Following are the steps taken for the assay,

1. Blood sample was taken from healthy human being who has not taken any NSAID for two weeks before the experiment.
2. Blood sample was transferred to heparinized centrifuge tubes and centrifuges at 3000 rpm for 15 minutes at room temperature.
3. The supernatant was removed with care and the red blood cell packs at the bottom were washed three to five times with normal saline (0.85% NaCl).
4. Human red blood cell suspension (10% v/v) was prepared in normal saline.
5. Different dilutions of plants' extracts and aspirin which was used as a control were prepared.
6. Reaction mixture was prepared with 2 ml normal saline, 0.5 ml of 10% v/v human red blood cell suspension and 1 ml of extract dilutions.
7. This reaction mixture was incubated at 37 0C for 30 minutes and then centrifuged at 3000 rpm for 20 minutes.
8. Using a spectrophotometer, the absorbance of the supernatant solution was determined at 560 nm.

9. The experiment was conducted in triplicates and mean average was taken.

The following equation was used to compute the % inhibition of haemolysis or membrane stabilisation.

% Inhibition of haemolysis = 100 x (A1-A2/A1)

Where:

A1 = Absorption of hypotonic buffered saline solution alone

A2 = Absorption of test sample in hypotonic solution

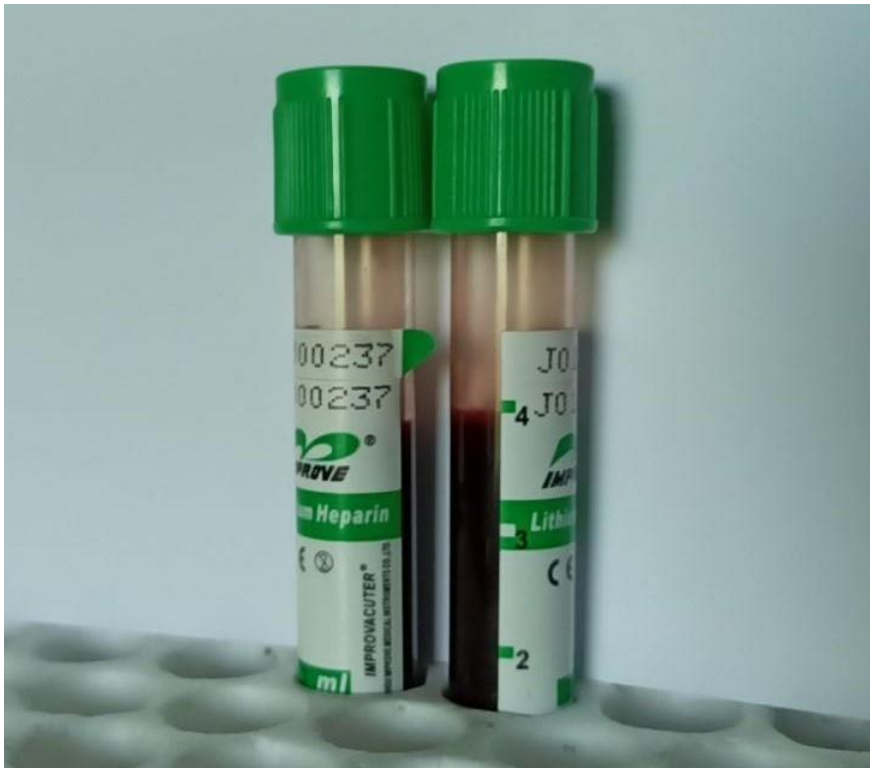


**Figure 2.6 Blood sample taken for membrane stabilization assay**

**Figure 3.7 The reaction mixtures being agitated in a centrifuge**

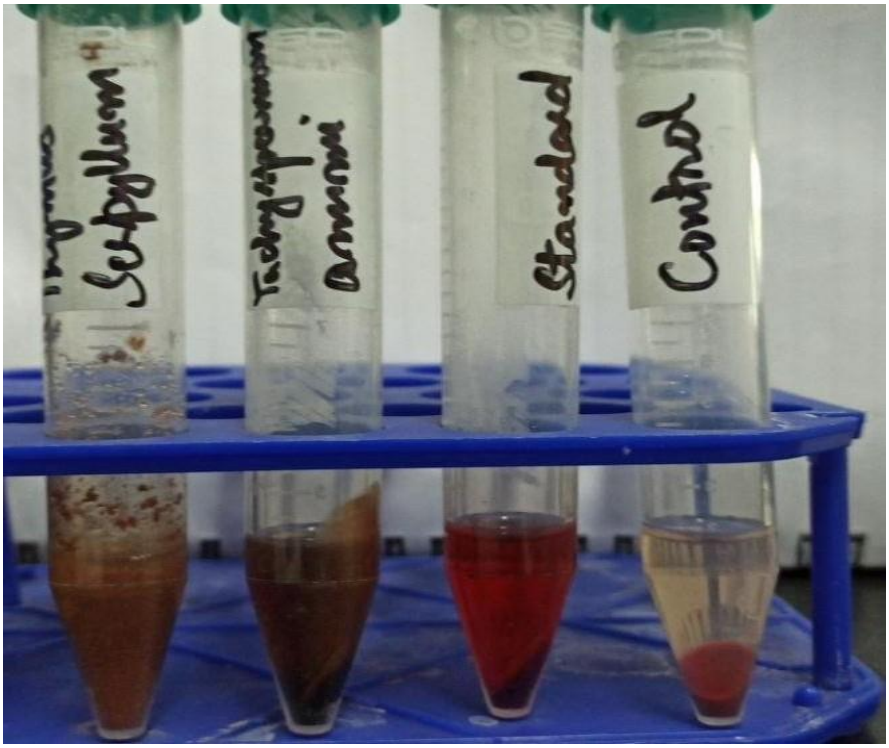


**Figure 3.8 Pellet formed after centrifugation of the reaction mixtures**

# Results

## 3.4 Evaluation of Drug-like Properties

A total of 763 compounds were found in methanolic extract of *Thymus serpyllum* whereas 937 compounds were found in methanolic extract of *Tachyspermum ammi*. These compounds were then scrutinized for their drug-like properties. In order to check the drug-like characteristics; Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule were considered. SwissADME online server predicted that only 135 compounds of T. ammi and 69 compounds of T. serpyllum followed all of the above mentioned rules. These compounds were then be elucidated for ADMET properties, among which Blood Brain Barrier (BBB), Human Intestinal Absorption (HIA), P-glucoprotein (Pgp) substrate, Human Ether-a-go-go-Related Gene Inhibition (HERG), AMES mutagenesis, Carcinogenicity were considered important parameters. BOILED Egg plot and ADMETsar online tool exhibited that out of these compounds only 10 compounds of T. ammi and 3 compounds of T. serpyllum showed all of these parameters correct.

**Table 4.1. Shortlisted phytochemicals on the basis of Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule**

| S. No. | T. ammi phytochemicals | T. serpyllum phytochemicals |
|---|---|---|
| 1 | Ethyl 2-(2-chloroacetamido)-3,3,3-trifluorolactate | 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride |
| 2 | 4-(1H-[1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester | Androst-4,6-dien-3,11,17-trione, 9-mercapto- |
| 3 | S-2-[2-Norbornylamino]ethyl thiosulfuric acid | Erythronolide A, 12-deoxy- |
| 4 | 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) | |
| 5 | Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester | |
| 6 | Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenyl-piperidin-3-yl ester | |
| 7 | 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone | |
| 8 | Xylose, 4-acetamido-4,5-dideoxy-, diethyl mercaptal, D- | |
| 9 | 1,3-Adamantanediacetamide | |
| 10 | Acetamide, N-methyl-N-[4-[4-[2-hydroxyethyl]-1-piperidyl]-2-butynyl]- | |

**Table 4.2. Different parameters upon which Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule helped shortlisting tahyspermum ammi compounds for drug-like properties (retrieved from SwissADME)**

| IUPAC Name | MOL. WT. | HBD | HBA | RB | TPSA | MlogP |
|---|---|---|---|---|---|---|
| Ethyl 2-(2-chloroacetamido)-3,3,3-trifluorolactate | 263.60 g/mol | 2 | 7 | 7 | 75.63 Å² | 0.17 |
| 4-(1H-[1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester | 253.26 g/mol | 1 | 5 | 5 | 91.42 Å² | -0.81 |
| S-2-[2-Norbornylamino]ethyl thiosulfuric acid | 251.37 g/mol | 2 | 4 | 5 | 100.08 Å² | 0.86 |
| 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) | 420.54 g/mol | 0 | 8 | 2 | 73.84 Å² | -0.66 |
| Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester | 264.32 g/mol | 1 | 5 | 4 | 53.01 Å² | 0.98 |
| Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenyl-piperidin-3-yl ester | 263.29 g/mol | 1 | 4 | 3 | 66.84 Å² | 0.76 |
| 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone | 346.37 g/mol | 3 | 6 | 1 | 104.06 Å² | 1.66 |
| Xylose, 4-acetamido-4,5-dideoxy-, diethyl mercaptal, D- | 281.44 g/mol | 3 | 3 | 9 | 120.16 Å² | 0.73 |
| 1,3-Adamantanediacetamide | 250.34 g/mol | 2 | 2 | 4 | 86.18 Å² | 1.46 |
| Acetamide, N-methyl-N-[4-[4-[2-hydroxyethyl]-1-piperidyl]-2-butynyl]- | 252.35 g/mol | 1 | 3 | 5 | 43.78 Å² | 1.05 |

**Table 4.3. Different parameters upon which Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule helped shortlisting thymus serpyllum compounds for drug-like properties (retrieved from SwissADME)**

| Compound | Mol. Wt. | HBA | HBD | Rotatable Bonds | TPSA | MlogP |
|---|---|---|---|---|---|---|
| 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride | 317.69 g/mol | 7 | 1 | 3 | 96.79 Å² | 1.41 |
| Androst-4,6-dien-3,11,17-trione, 9-mercapto- | 330.44 g/mol | 3 | 0 | 0 | 90.01 Å² | 2.44 |
| Erythronolide A, 12-deoxy- | 402.52 g/mol | 7 | 4 | 1 | 124.29 Å² | 1 |

**Table 4.4. Different parameters of ADMET properties upon which ADMETsar evaluated *Tachyspermum ammi* compounds for pharmacokinetics properties.**

| IUPAC Name | Blood-Brain Barrier | Human Intestinal Absorption | Human Oral Bioavailability | Ames Toxicity | Human Ether-A-Go-Go-Related Gene Inhibition | Hepatotoxicity |
|---|---|---|---|---|---|---|
| Ideal case | -ive | +ive | +ive | -ive | -ive | -ive |
| Ethyl 2-(2-chloroacetamido)-3,3,3-trifluorolactate | -ive | +ive | +ive | -ive | -ive | -ive |
| 4-(1H-[1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester | -ive | +ive | +ive | -ive | -ive | -ive |
| S-2-[2-Norbornylamino]ethyl thiosulfuric acid | -ive | +ive | +ive | -ive | -ive | -ive |
| 3,3'-Isopropylidenebis(1,5,8,11-tetraoxacyclotridecane) | -ive | +ive | +ive | -ive | -ive | -ive |
| Benzoic acid, 2-hydroxy-5-(4-methyl-1-piperazinyl)methyl-, methyl ester | -ive | +ive | +ive | -ive | -ive | -ive |
| Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenyl-piperidin-3-yl ester | -ive | +ive | +ive | -ive | -ive | -ive |
| 2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene 1,10-carboxylic acid 1-4 lactone | -ive | +ive | +ive | -ive | -ive | -ive |
| Xylose, 4-acetamido-4,5-dideoxy-, diethyl mercaptal, D- | -ive | +ive | +ive | -ive | -ive | -ive |
| 1,3-Adamantanediacetamide | -ive | +ive | +ive | -ive | -ive | -ive |
| Acetamide, N-methyl-N-[4-[4-[2-hydroxyethyl]-1-piperidyl]-2-butynyl]- | -ive | +ive | +ive | -ive | -ive | -ive |

**Table 4.5. Different parameters of ADMET properties upon which ADMETsar evaluated *Thymus serpyllum* compounds for pharmacokinetics properties.**

| IUPAC Name | Blood-Brain Barrier | Human Intestinal Absorption | Human Oral Bioavailability | AMES Toxicity | Human Ether-a-go-go-Related Gene Inhibition | Hepatotoxicity |
|---|---|---|---|---|---|---|
| **Ideal case** | **-ive** | **+ive** | **+ive** | **-ive** | **-ive** | **-ive** |
| 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride | -ive | -ive | -ive | -ive | -ive | -ive |
| Androst-4,6-dien-3,11,17-trione, 9-mercapto- | -ive | -ive | -ive | -ive | -ive | -ive |
| Erythronolide A, 12-deoxy- | -ive | -ive | -ive | -ive | -ive | -ive |

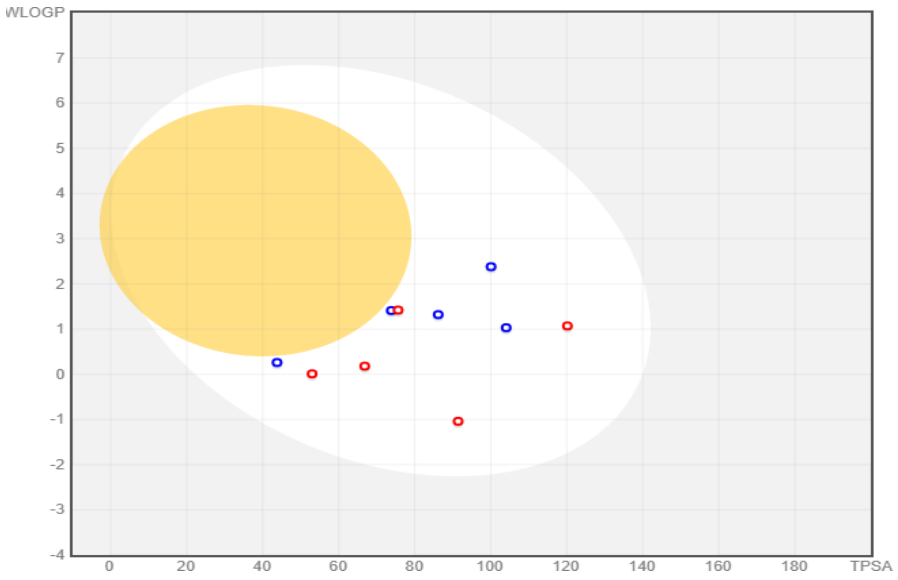


**Figure 4.2. BOILED-EGG plot of top 10 phytochemicals of *Tachyspermum ammi* with best drug-like properties**

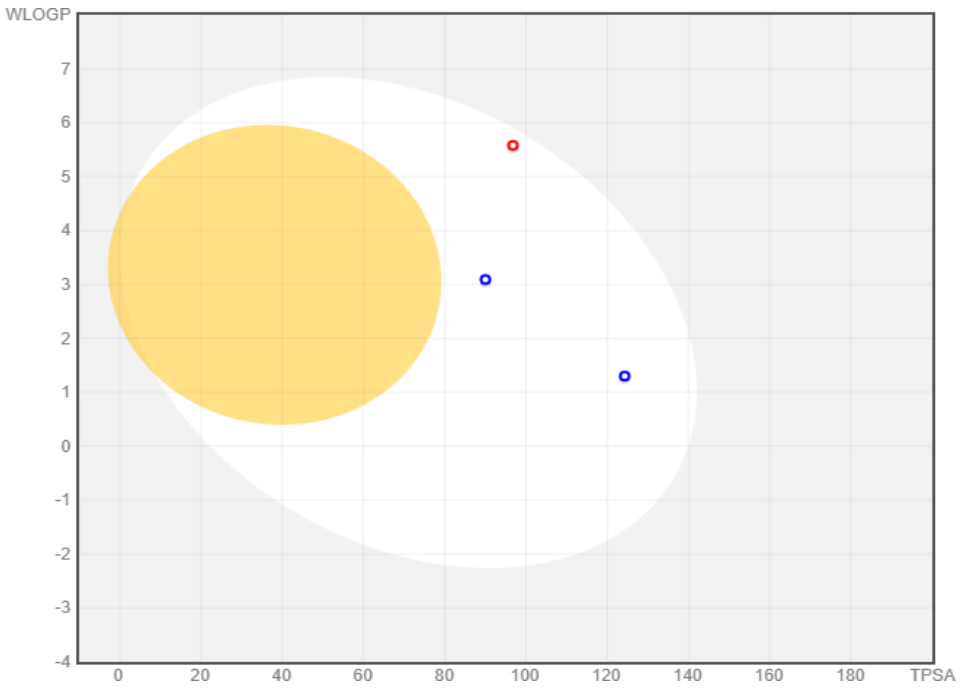


**Figure 4.3. BOILED-EGG plot of top 3 phytochemicals of *Thymus serpyllum* with best drug-like properties**

## 3.5 HUB Gene Analysis

The hub gene analysis performed through Cytoscape's extension known as cytoHubba resulted in top 10 hub genes of rheumatoid arthritis by applying different criteria. Only top two hub genes from this list were taken for further *in-silico* analysis.

**Table 4.6 Top 10 hub genes calculated through cytohubba with the top two hub genes highlighted which were taken for further *in-silico* analysis**

| Eccentricity | MNC | MCC | DEGREE | EPC |
|---|---|---|---|---|
| TNF | TNF | TNF | TNF | IL6 |
| CD40LG | CD40LG | CD40LG | ALB | TNF |
| VEGFA | VEGFA | VEGFA | VEGFA | IL1B |
| TLR2 | TLR2 | TLR2 | TLR2 | IFNG |
| CSF2 | CSF2 | CSF2 | CSF2 | TLR2 |
| CD80 | CD80 | CD80 | TLR1 | VEGFA |
| TLR1 | TLR1 | TLR1 | IFNG | CSF2 |
| IFNG | IFNG | IFNG | JUN | TLR1 |
| IL6 | IL6 | IL6 | IL6 | CD40LG |
| IL1B | IL1B | IL1B | IL1B | ALB |

The top two hub genes reruited from hub gene analysis were Vascular endothelial growth factor A (VEGFA) and Tumor necrosis factor alpha (TNF- α) which were used further for the molecular docking.

### 3.6 Molecular Docking

Top compounds that were short-listed in previous step were then used for molecular docking analysis through MOE software. For docking study, Vascular endothelial growth factor A (VEGFA) and Tumor necrosis factor alpha (TNF- **α)** taken as target proteins. Three dimensional structures of target proteins were available on Protein Data Bank (PDB) therefore, they were downloaded in PDB format. Figure 4.5 shows the 3-D structures of both target proteins.

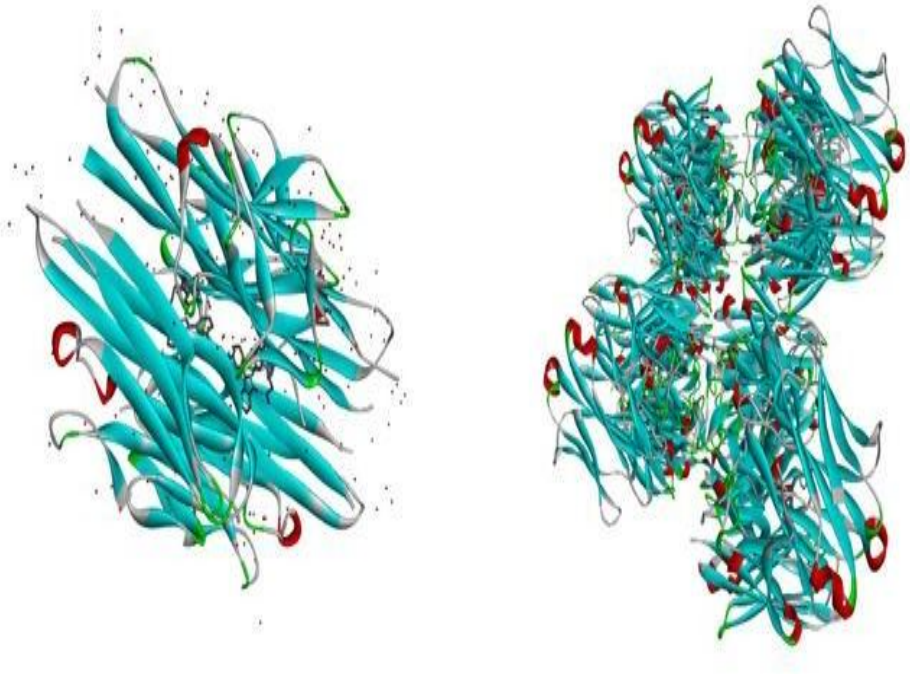

**Figure 4.4. 3D structure of TNF alpha (left) and VEGFA (right) as downloaded from PDB without cleaning**

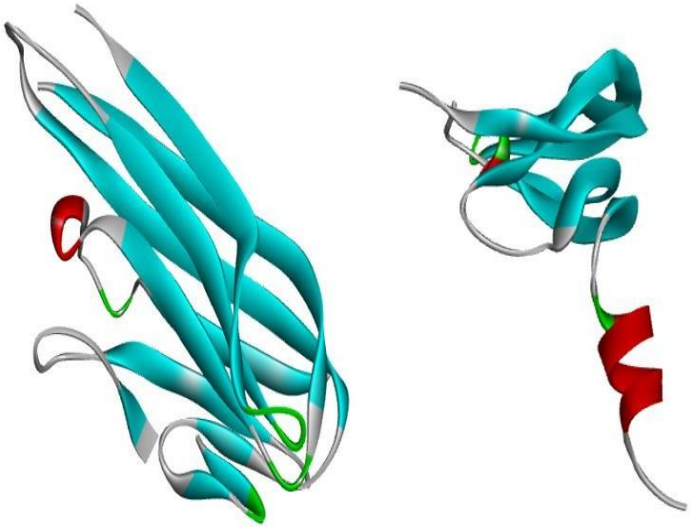

**Figure 4.5. 3-D structure of TNF alpha (left) and VEGFA (right) after cleaning and removing water and other extra molecules using MOE software**

Table 4.7 shows the compounds which exhibited best docking score among all shortlisted compounds of Tachyspermum ammi and Thymus serpyllum with TNF alpha and VEGFA alongwith their rmsd refine values. The lesser the value of binding energy, stronger is the binding between ligand and protein.

**Table 4.7 Moe results of docking analysis of test compounds against TNF alpha and VEGFA**

| Plant | Ligand | Ligand's Pubchem ID | Protein | Binding Energy (Kcal/ Mol) | rmsd_ refine | Sites | Regions |
|---|---|---|---|---|---|---|---|
| *Tachyspermum ammi* | Methyl 2-hydroxy-5-[(4-methylpiperazin-1-yl)methyl]benzoate | 567259 | TNF | -8.0028 | 1.9483 | 134 | Beta strand region |
| | S-2-[2-Norbornylaminoethyl thiosulfuric acid | 579994 | VEGFA | -8.0039 | 1.2390 | 64 | Disulfide |
| *Thymus serrulum* | 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride | 2777526 | VEGFA | -7.0656 | 2.2639 | 104 | Beta strand region |
| | Erythronolide A, 12-deoxy | 102956 | TNF | -8.5186 | 1.9138 | 45 | Transmembrane region<br>Helical region |

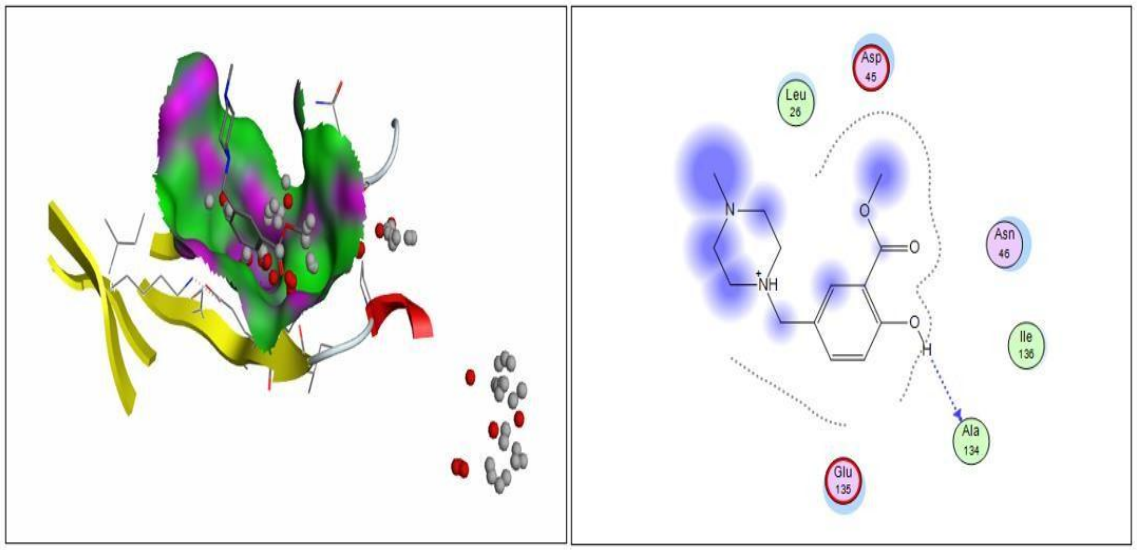


**Figure 4.6. 3-dimensional (left) and 2-dimensional (right) interaction of Methyl 2-hydroxy-5-[(4-methylpiperazin-1-yl)methyl]benzoate with the binding pockets of TNF alpha protein**

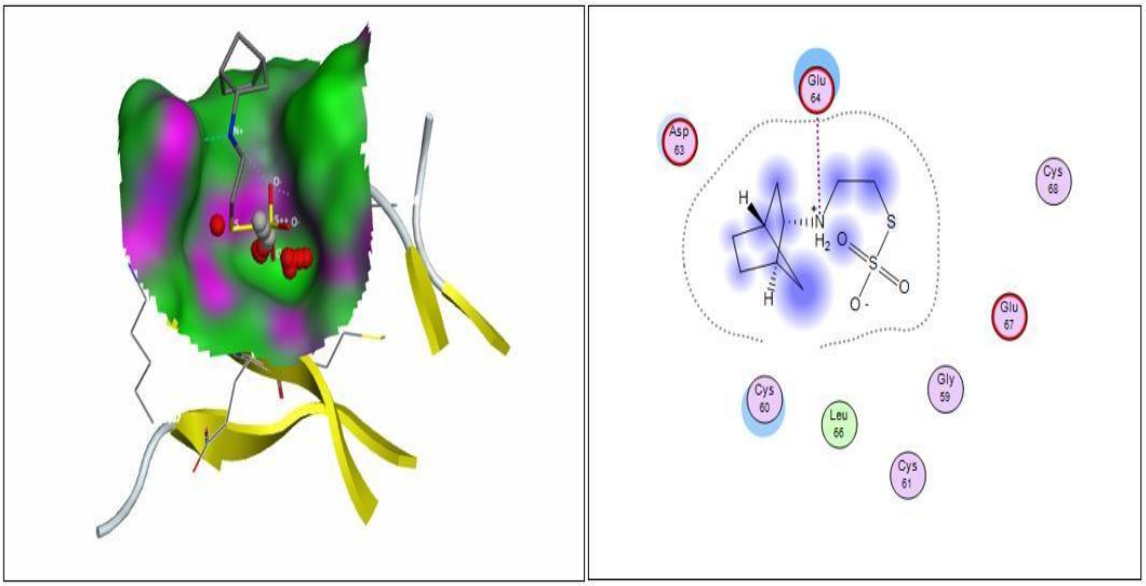


**Figure 4.7.. 3-dimensional (left) and 2-dimensional (right) interaction of S-2- [2- Norbornylamino]ethyl thiosulfuric acid with the binding pockets of VEGFA protein**

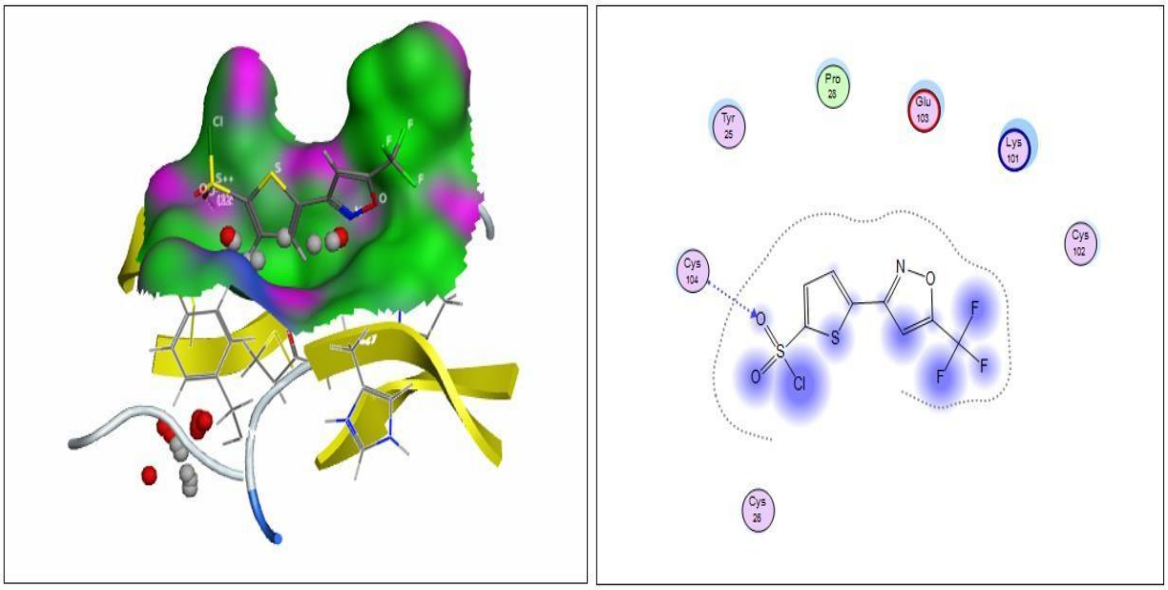


**Figure 4.8. 3-dimensional (left) and 2-dimensional (right) interaction of 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride with the binding pockets of VEGFA protein**

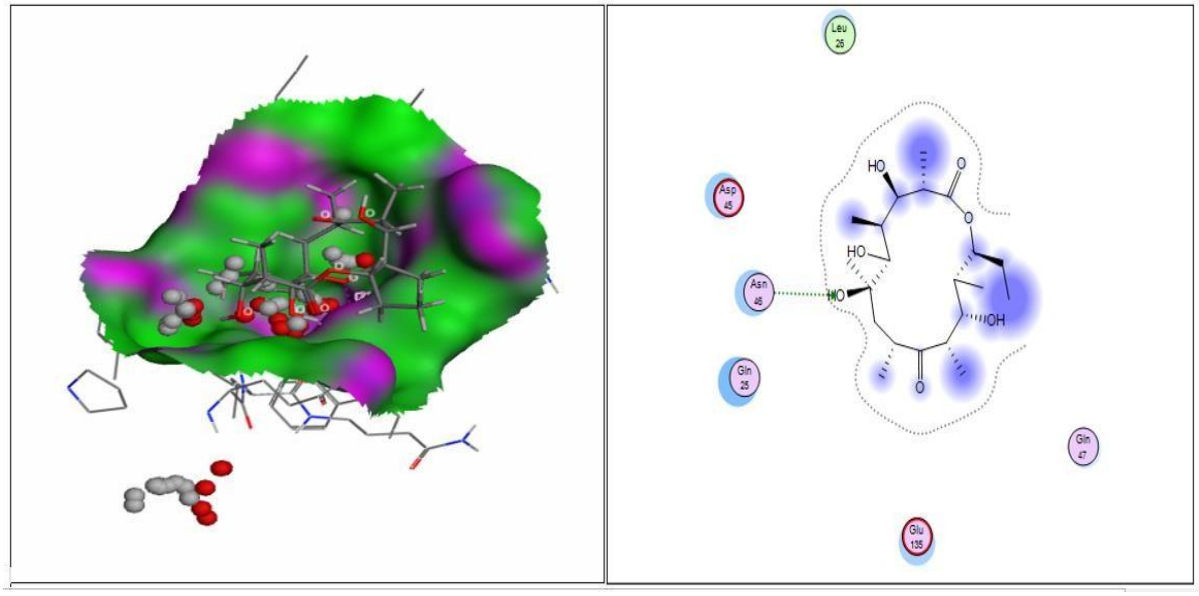


**Figure 4.9. 3-dimensional (left) and 2-dimensional (right) interaction of Erythronolide A, 12-deoxy with the binding pockets of TNF alpha protein**

## 3.7 In-vitro analysis

### 3.7.1 Phytochemical testing

Phytochemical testing was done on aqueous and methanolic extracts of *Thymus serpyllum* and *Tachyspermum ammi* as described in chapter 3. The results of both extracts are summarized in following tables.

**Table 4.8 Phytochemical testing results of *Tachyspermum ammi***

| Test name | Aqueous extract | Methanolic extract |
|---|---|---|
| Alkaloids | -ve | +ve |
| Anthocyanins | +ve | +ve |
| Tannins | +ve | +ve |
| Terpenoids | +ve | +ve |
| Phenols | +ve | +ve |
| Flavinoids | -ve | +ve |

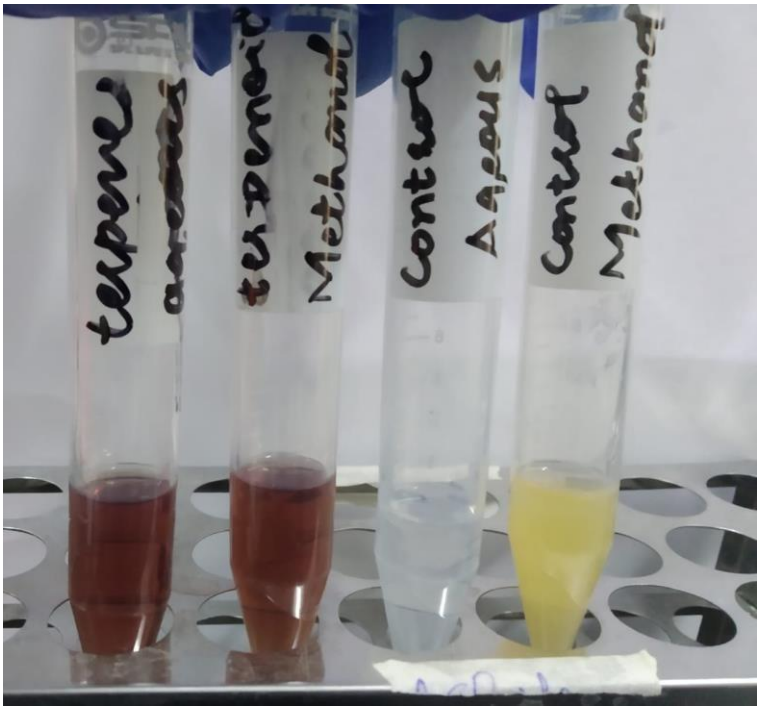

**Figure 3.10 Test results of terpenoids in *Tachyspermum ammi***

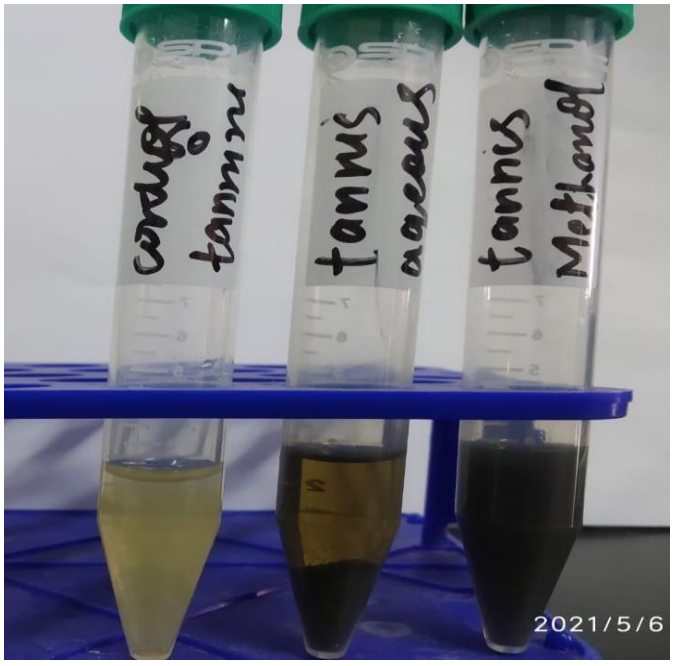


**Figure 4.11 Test results of tannins in *Tachyspermum ammi***

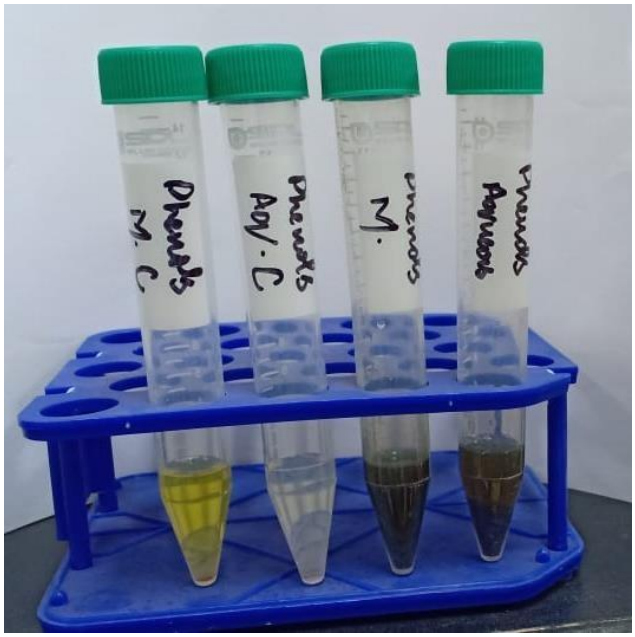

**Figure 4.12 Test results of phenols in *Tachyspermum ammi***

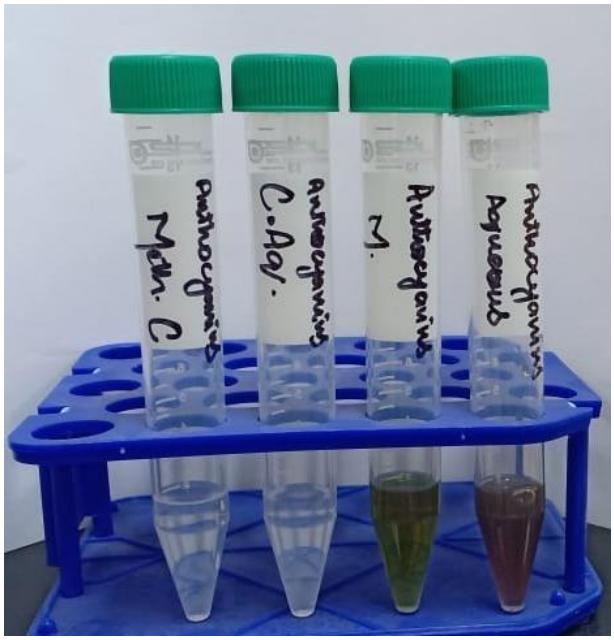

**Figure 4.13 Test results of anthrocyanins in Tachyspermum ammi**

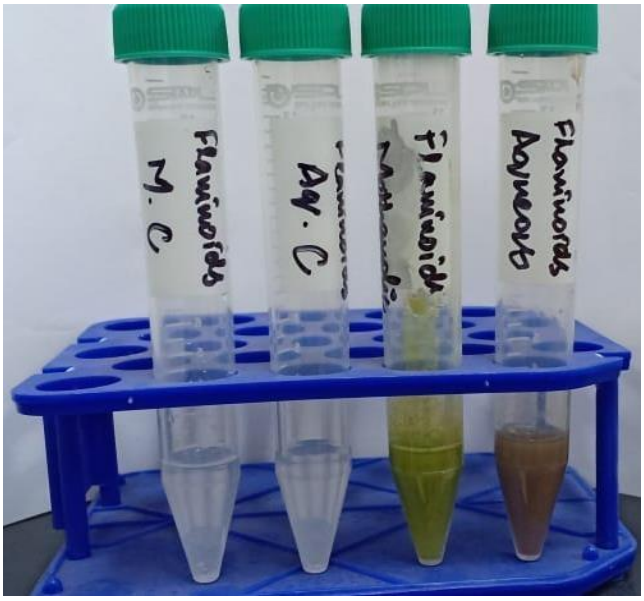


**Figure 4.14 Test results of flavonoids in Tachyspermum ammi**

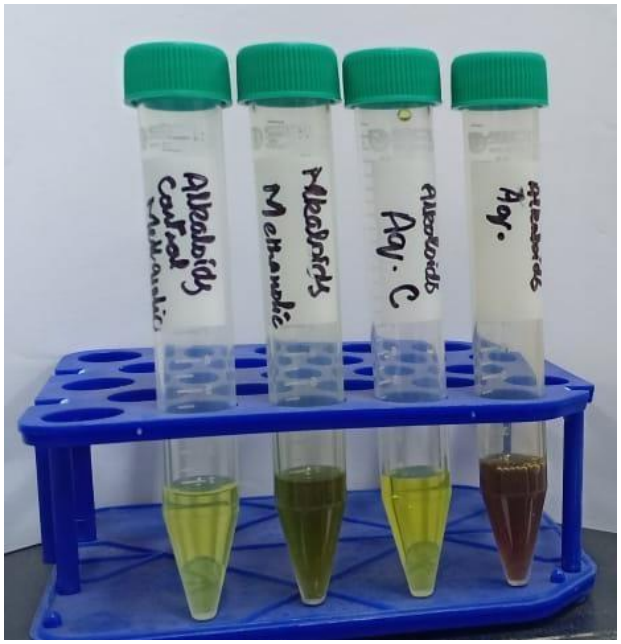


**Figure 4.15 Test results of alkaloids in *Tachyspermum ammi***

**Table 4.9 Phytochemical testing results of *Thymus serpyllum***

| Test name | Aqueous extract | Methanolic extract |
|---|---|---|
| Alkaloids | -ve | +ve |
| Tannins | +ve | +ve |
| Anthocyanins | +ve | +ve |
| Terpenoids | +ve | +ve |
| Phenols | +ve | +ve |
| Flavinoids | +ve | +ve |

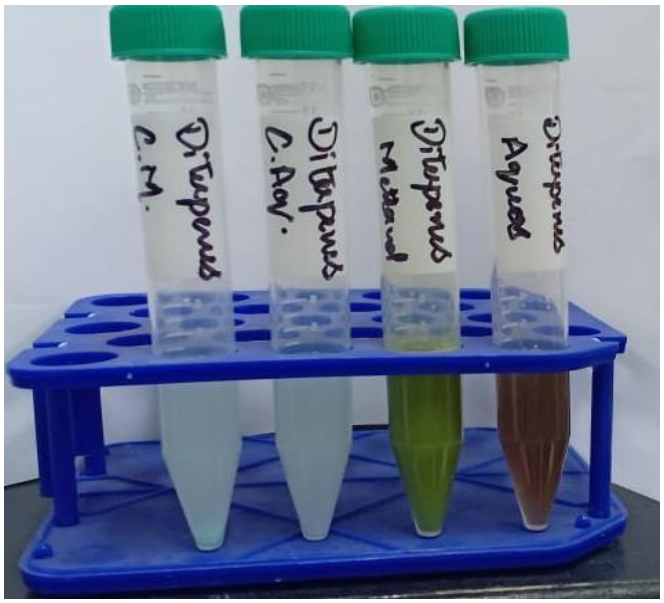


**Figure 4.16 Test results of terpenoids in *Thymus serpyllum***

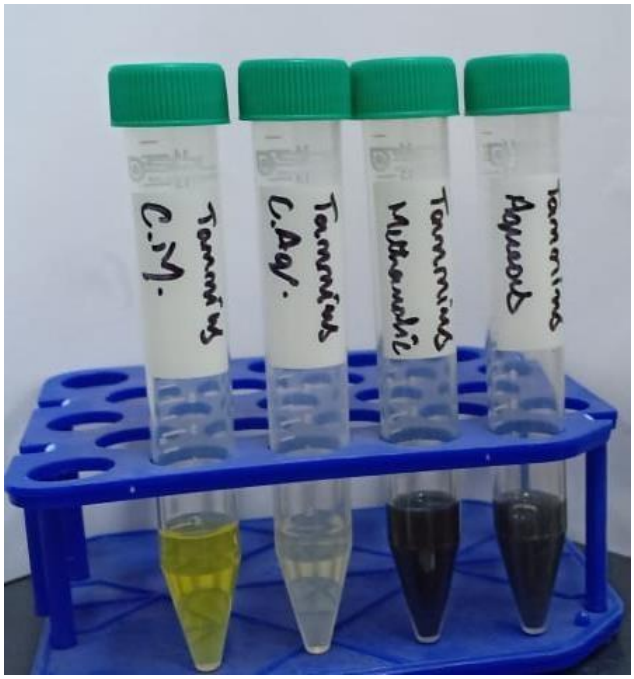

**Figure 4.17 Test results of tannins in *Thymus serpyllum***

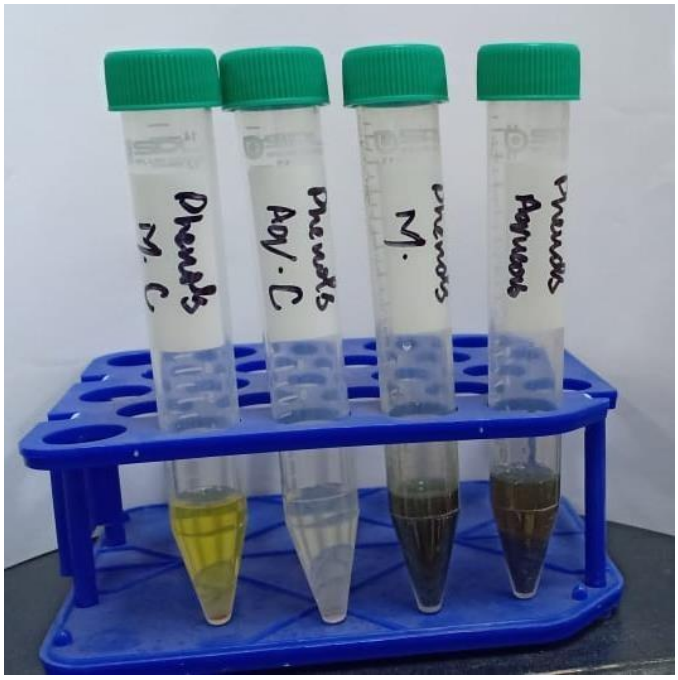

**Figure 4.18 Test results of phenols in *Thymus serpyllum***

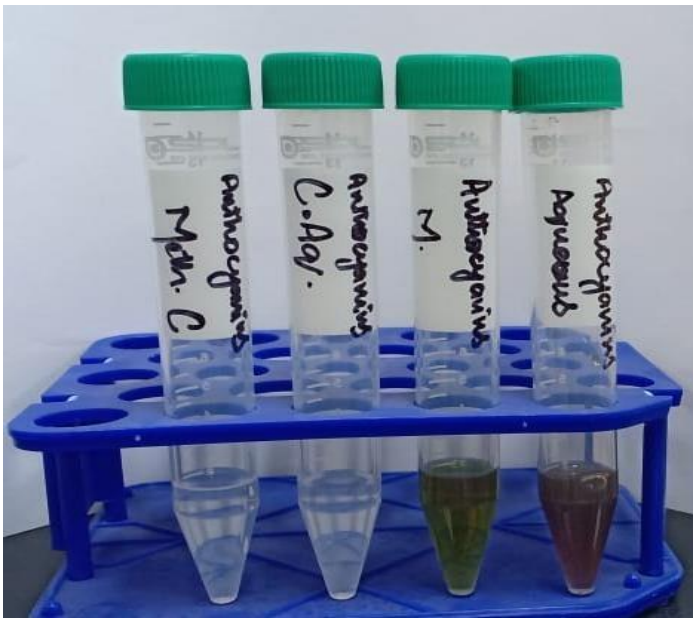

**Figure 4.19 Test results of anthrocyanins in *Thymus serpyllum***

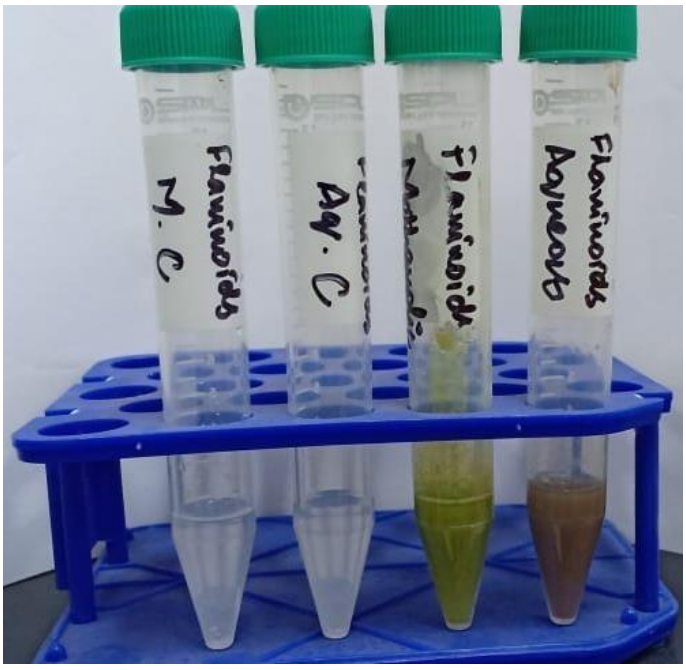

**Figure 4.20 Test results of flavonoids in *Thymus serpyllum***

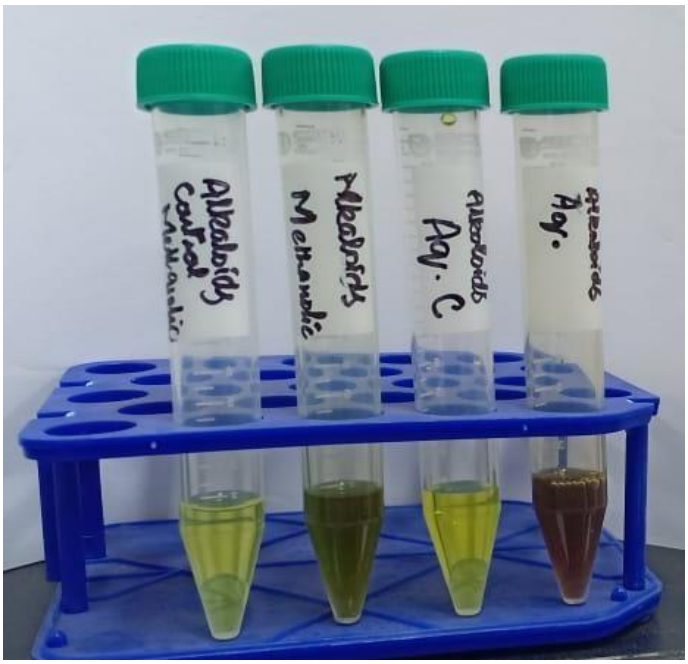

**Figure 4.21 Test results of alkaloids in *Thymus serpyllum***

### 3.7.2 DPPH Assay

The anti-oxidant activity of both plants' extracts were calculated through DPPH assay. Anti-oxidant activity of both control (ascorbic acid) and plants' extracts were observed by their free radical scavenging activity. The radical scavenging potential increased with increasing dosage in case of both tachyspermum ammi and thymus sepyllum extracts just like that of the control (ascorbic acid), but thymus serpyllum exhibited more potent radical scavenging activity than that of trachyspermum ammi. Table 4.10 shows the inhibition percentage of test samples and the figure 4.22 shows the graphical representation of increasing gradient.

**Table 4.10 Perecentage inhibition by compounds in DPPH assay**

| Conc. (µg/mL) | 20 | 40 | 60 | 80 | 100 |
|---|---|---|---|---|---|
| Ascorbic acid | 77% | 85% | 86% | 86% | 87% |
| *T. serpyllum* (M) | 78% | 81% | 85% | 88% | 93% |
| *T. ammi* (M) | 75% | 81% | 83% | 84% | 87% |

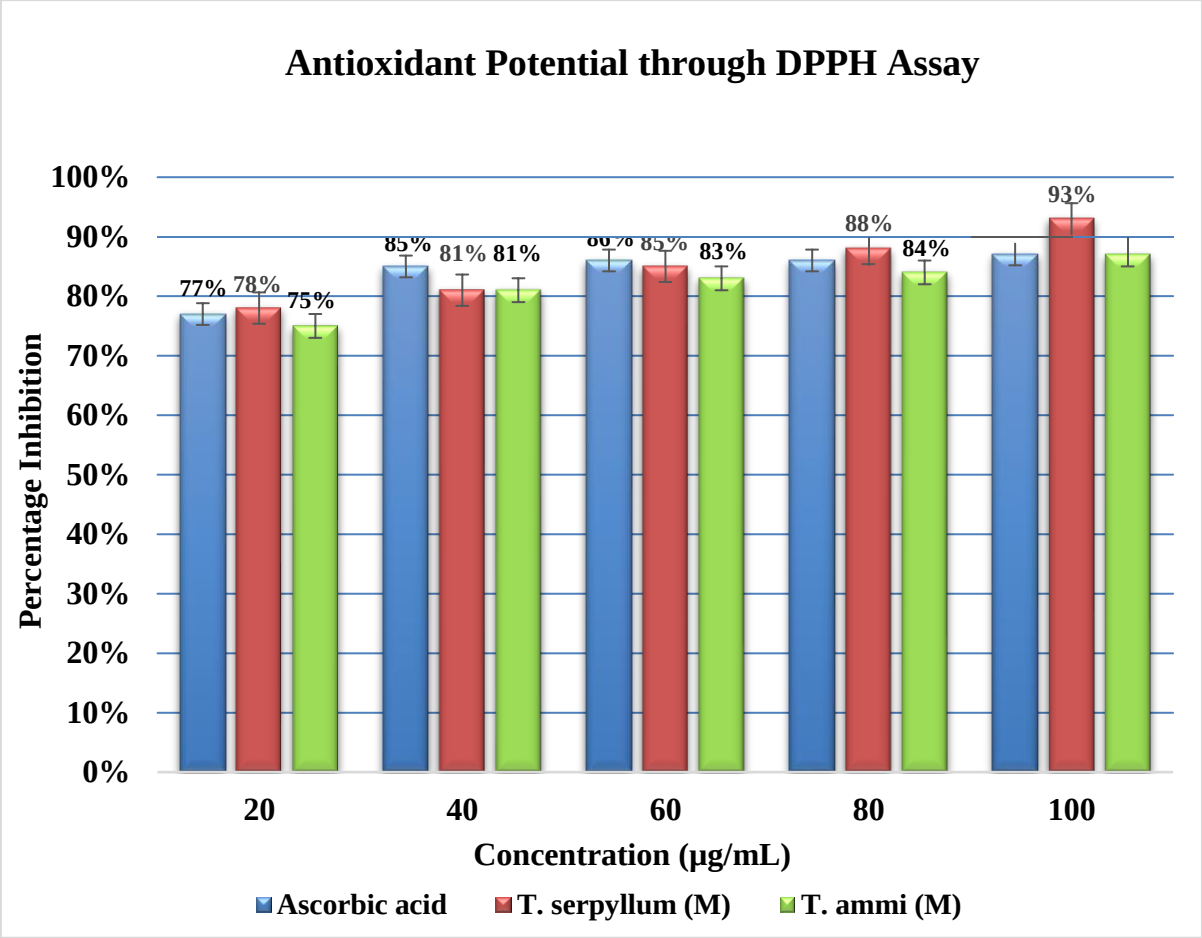


**Figure 4.22 graphical representation of DPPH assay results**

### 3.7.3 Albumin denaturation

Albumin denaturation assay works on the principle of protein denaturation. Higher the capacity of a compound to help resist protein denaturation, higher is the anti-inflammatory potential of that compound. The results suggested that both trachyspermum ammi and thymus serpyllum have shown great percentage inhibition with increasing potentials in comparison with the standard drug i.e. ibuprofen. Furthermore, thymus serpyllum indicated a greater potential towards inhibition of protein denaturation than that of tracchyspermum ammi.

**Table 4.11 Percentage inhibition by compounds in albumin denaturation assay**

| **Conc. (µg/mL)** | 200 | 400 | 600 | 800 | 1000 |
|---|---|---|---|---|---|
| **Ibuprofen** | 32% | 39% | 42% | 49% | 53% |
| ***T. ammi* (M)** | 71% | 75% | 81% | 85% | 92% |
| ***T. serpyllum* (M)** | 72% | 78% | 83% | 88% | 97% |

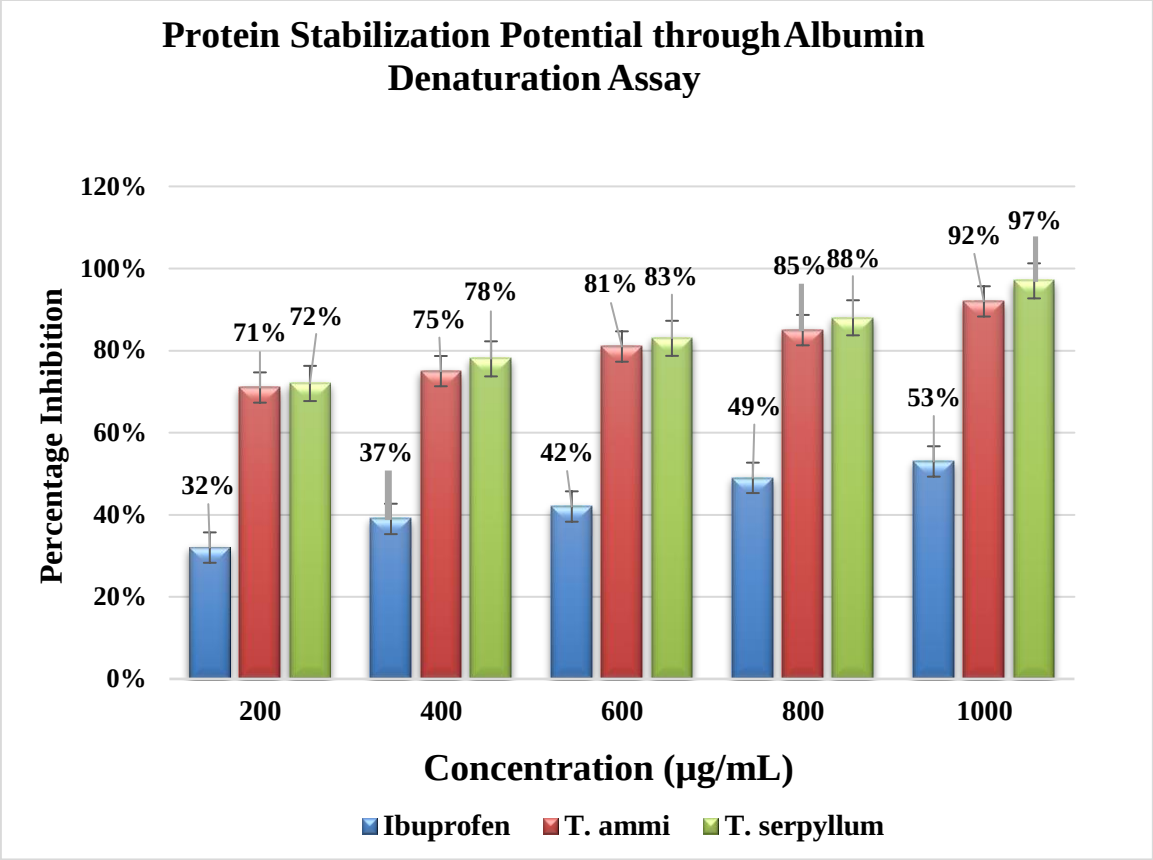


**Figure 4.23 Graphical representation of albumin denaturation assay**

### 3.7.4 HRBC Membrane Stabilization Assay

Membrane stabilization assay suggested the potential of a compound to stabilize red blood cell membrane which mimics the lysosomal membrane. During arthritis, lysosomal membrane is broken down and many harmful enzymes are released which cause damage to other organelles as well. The results obtained from this assay suggested that thymus serpyllum exhibited greater membrane stabilizing activity than that of trachyspermum ammi, where they both were evaluated against aspirin taken as a control.

**Table 4.12 Percentage inhibition by compounds in membrane stabilization assay**

| Conc. (µg/mL) | 200 | 400 | 600 | 800 | 1000 |
|---|---|---|---|---|---|
| **Aspirin** | 44% | 58% | 81% | 92% | 97% |
| ***T. serpyllum* (M)** | 49% | 57% | 69% | 79% | 95% |
| ***T. ammi* (M)** | 26% | 49% | 65% | 76% | 90% |

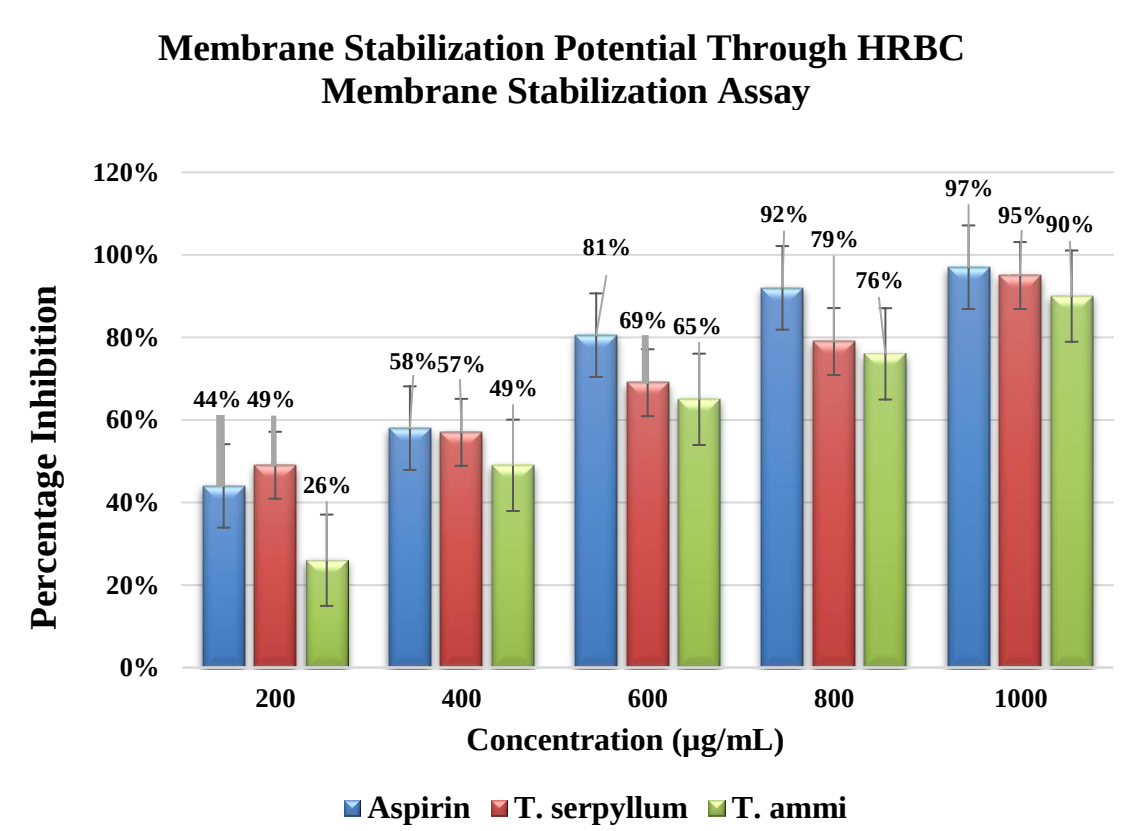


**Figure 4.24 Graphial representation of HRBC membrane stabilization assay**

# Discussion

Rheumatoid Arthritis (RA) is an autoimmune arthropathic illness that affects people all over the world (Firestein 2003) and is characterised by diarthrodial joint inflammation, symmetrical polyarthritis, and synovial hyperplasia (swelling), which leads to progressive bone and cartilage degeneration, loss of articular function, and final joint deformity (Guo, Wang et al. 2018). Although the exact etiological milieu for RA is unknown, a variety of endogenic and exogenic triggers, as well as genetic predispositions, appear to be associated to increasing autoimmune reactions in the synovial membrane (Edwards, Szczepański et al. 2004). ) T cells and B cells both play a role in the pathogenesis of RA, with pro-inflammatory cytokines playing a significant role. T and B lymphocyte activation activates a loop mechanism that allows T cells, macrophages, and B cells to interact in new ways (Smolen and Steiner 2003, Smolen, Aletaha et al. 2007). Macrophages, especially, are vital synovitis effectors that work through antigen presentation and phagocytosis, as well as the matrix-degrading enzymes, generation of reactive oxygen species (ROS), proinflammatory cytokines (IL-6, TNF alpha and IL-1), nitrogen intermediates and prostanoids (McInnes & Schett, 2011).

There is no definitive diagnostic method that can confirm the presence of RA. A full blood profile, differential, rheumatoid factor (RF), and C-reactive protein (CRP) or erythrocyte sedimentation rate can all be included in the initial lab testing (ESR) (Kwoh, Anderson et al. 2002). Nonsteroidal anti-inflammatory medications (NSAIDs), glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and biologics are among the current therapeutic choices for RA patients. NSAIDs such as celecoxib, indomethacin acetic acid, aspirin, naproxen, and ibuprofen have an analgesic effect and

lead to numerous side effects such as cardiovascular risk, gastro-intestinal issues, and renal dysfunction (Mitragotri and Yoo 2011). Anti-inflammatory mediators such as dexamethasone and prednisolone inhibit phospholipid release, resulting in a reduction in articular inflammation, but cause undesirable consequences such as cardiovascular diseases, osteoporosis, poor glucose metabolism (insulin resistance), skin thinning, hypertension, decreased lesion healing, and obesity in the long run (Hoes, Jacobs et al. 2010, Mitragotri and Yoo 2011). DMARDs like methotrexate slow down the progression of RA and decrease articular degeneration (McInnes and Schett 2011). Interstitial pneumonitis, myelosuppression, hepatic cirrhosis, retinopathies, hypersensitivity, and allergic responses are also severe side effects of DMARDs (von Vollenohower 2009).

Phytochemicals are biologically active constituents of plants that are extracted directly from them (Georgiev 2014). Numerous epidemiological studies have found that consuming phytochemicals on a regular basis is linked to a lower risk of different malignancies (Russo, Spagnuolo et al. 2010). *Thymus Serpyllum* (wild Thyme) is a herbal plant that is widely utilised for its antibacterial properties and beneficial benefits on the human digestive system. All of the necessary therapeutic qualities are present in this wild thyme (Grieve 2013). *Trachyspermum ammi*, popularly known as Ajwain, is very medicinal and broncho-dilating, antihypertensive, antispasmodic, hepatoprotective (Gilani, Jabeen et al. 2005), anti-aggregant (Srivastava and Acids 1988), antitussive (Boskabady, Jandaghi et al. 2005), anti-aflatoxin (Anilakumar, Saritha et al. 2009) properties.

In silico analysis and bioinformatics have completely transformed the way drugs are designed. It has reduced both the cost and the time spent on medication development.

Compounds may be evaluated for their characteristics and capacity to work as a drug in the human body using contemporary computational techniques (Wadood, Ahmed et al. 2013). The purpose of this study was to identify the lead phytochemicals with minimum side effects, which could be used as a future anti-arthritic drug. The first step to achieve this purpose was the in silico assessment of drug's properties via SwissADME on the basis of five different rules namely; Lipinski rule of five, Ghose rule, Muegge rule, Egan rule and Veber rule (Bathen and Linder 2017). 135 of the total compounds found in *T. ammi* and 69 in *T. serpyllum* followed these 5 rules and upon further elucidation of their ADMTE parameters, only 10 in *T. ammi* and 3 in *T. serpyllum* fulfilled these parameters.

For docking analysis TNF alpha and VEGFA were taken as hub genes of arthritis and their 3D structures were obtained from Protein Data Bank (PDB). 3D structures of TNF and VEGFA were then docked with libraries containing 3D structures of phytochemicals of both plants by means of "Molecular Operating Environment" (MOE) software (Roy, Luck et al. 2007). Compounds having minimum binding energy are shown to be most tightly bound to these proteins with their binding regions having their own significance.

Air dried extracts of both plants were obtained after dissolving plants in ethanol and water, separately, and were filtered after being kept in a dark chamber for 10 days. Phytochemical testing was done on aqueous and ethanolic extracts which showed both extracts to be positive for phenols, tannins, terpenoids and anthocyanins while aqueous extract was found to be negative for alkaloids and flavinoids in case of *T. ammi* extract while the aqueous extract of *T. serpyllum* gave negative results for alkaloids while all other phytochemical tests gave positive results in both aqueous and ethanolic extracts.

DPPH assay was performed to check the antioxidant potential of ethanolic extracts of both plants and the procedure was a bit modified than (Sanganna, Chitme et al. 2016). Radical scavenging potential of extracts from both plants increased with increasing the dosage of extracts but it was found to be relatively higher in the extract of *T. serpyllum* than that of *T. ammi* in comparison to the standard, ascorbic acid.

Albumin denaturation assay was done with slight changes in (Chandra, Chatterjee et al. 2012). Both plant extracts showed great percentage inhibition with increasing potentials as relative to the standard, ibuprofen, and *T. serpyllum* extract was found to be more potent than that of *T. ammi.*

Slight modifications were done to (Shailesh, Seema et al. 2011) method for HRBC membrane stabilization assay and aspirin was taken as control. *T. serpyllum* extract was found to be more effective for membrane stabilization of Red blood cells as compared to the *T. ammi* extract along with the control.

Thus, in conclusion, both plants exhibited their anti-rheumati potential through in-silico and in-vitro analysis but among both of these *Thymus serpyllum* exhibited itself more potent towards the treatment of inflammation than that of *Trachyspermum ammi.*

# Conclusion

The study conducted revealed that thymus serpyllum possesses much more anti-arthriti potential than that of tachyspermum ammi. It also recognized compounds from these two medicinal plants which showed great binding energies with two hub genes i.e. TNF alpha and VEGF-A, which play a key role in development of rheumatoid arthritis. These compounds are Methyl 2-hydroxy-5-[(4-methylpiperazin-1-yl)methyl]benzoate and S-2-[2-Norbornylamino]ethyl thiosulfuric acid from tachyspermum ammi which exhibited greater binding forces with TNF alpha and VEGF-A respectively. Similarly, Erythronolide A, 12-deoxy and 5-[5-(Trifluoromethyl)isoxazol-3-yl]thiophene-2-sulfonyl chloride are the compounds of thymus serpyllum which showed great binding forces with TNF alpha and VEGF-A respectively. These compounds found from in-silico analysis may be further tested for their modes of action using in-vitro and in-vivo techniques for development of new potential drugs against rheumatoid arthritis.

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