# *In silico* study of chemical compounds present in *Syzygium cumini* for antiarthritic activity

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*Abstract-* **An inflammatory, autoimmune illness that progresses is rheumatoid arthritis (RA) that causes joint inflammation and progressive joint damage. The search for effective therapeutic options for rheumatoid arthritis (RA) has led to the investigation of natural compounds as potential drug leads. In this study, we employed a comprehensive molecular docking approach to identify potential drug leads targeting high-temperature requirement protein 1 (HtrA1), a key player in RA pathogenesis from the plant Syzygium cumini. Using the IMPPAT database, we collected 241 phytoconstituents of Syzygium cumini and performed blind docking with the HtrA1 protein (PDB ID: 3TJO). Three distinct active sites were identified, and the top-ranking compounds were selected based on binding energy. Among the compounds evaluated, 3-O-Acetyloleanolic acid, Betulinic acid, (3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid, Corilagin, and Friedelon exhibited promising binding interactions with HtrA1, suggesting their potential as lead candidates for RA.**

*Keywords***: Rheumatoid arthritis, High-Temperature Requirement Protein 1, Molecular docking, Virtual screening.**

## 1.        Introduction

Rheumatoid arthritis is an autoimmune disease, which manifests with symptoms such as pain, stiffness, swelling, and joint dysfunction (McInnes et al., 2011, Radu et al, 2021). It is a systemic condition that predominantly damages the synovial lining of the joints, causing pain, edema, stiffness, and gradual joint deterioration. RA affects approximately 1% of the global population, with a higher prevalence in women than men (Angum et al, 2020).

Although there is no known cure for arthritis, there are therapies that can lessen pain, enhance function, and halt the disease's development. Some of the treatment options for arthritis include medications such as pain relievers, anti-inflammatory drugs, and disease-modifying antirheumatic drugs (DMARDs), physical therapy, occupational therapy and surgery (Benzamin et al.,2018; Prasad et al, 2013).

Inflammatory pathways are crucial in the onset and development of arthritis (Chen et al, 2019). These pathways are responsible for recruiting immune cells to the site of inflammation, producing inflammatory mediators, and causing damage to joint tissues. There are many different inflammatory pathways involved in arthritis, but some of the most important ones include the toll-like receptor (TLR) pathway, the NF-κB pathway, and JAK-STAT pathway (Arleevskaya et al., 2020; Malemud et al., 2018). One of the proteins that is involved in the pathogenesis of RA is high-temperature requirement protein 1 (HtrA1) (Hou et al. 2014, Tossetta et al, 2022). HtrA1 is a serine protease that is expressed in a variety of tissues, including the joints. It is believed to have an impact in the degradation of extracellular matrix proteins, such as fibronectin and collagen. As a result, the joints' bone and cartilage may be destroyed, which is characteristic of RA. In addition to its role in extracellular matrix degradation, HtrA1 has also been shown to promote inflammation and joint destruction by activating other pro-inflammatory pathways. For example, the transcription factor NF-B, which is involved in the generation of inflammatory cytokines, can be activated by HtrA1. The increased expression of HtrA1 in RA synovial fluid and tissues suggests that it could be a potential therapeutic target for the disease (Garu et.al, 2006). Several studies have shown that targeting HtrA1 can reduce inflammation and joint destruction in animal models of RA.

Plants can be used to help manage the symptoms of arthritis. Some of the most commonly used plants for arthritis include turmeric, ginger, Boswellia, Devil's claw, and aloe vera (Ansari et. al, 2023). *Syzygium cuminii*, commonly referred to as jamun, is a well-recognized botanical species that has been traditionally employed in folk medicine due to its reputed anti-inflammatory characteristics. This plant's anti-inflammatory properties have been well studied in both lab tests and animal models (Kumar et al., 2008; Qamar et al., 2023).

The purpose of the current investigation was to the binding affinity of the bioactive constituents present in *Syzygium cuminii* with HtrA1 through molecular docking analysis. By employing molecular docking techniques, the interaction between the selected compounds and HtrA1 was assessed, providing valuable insights into their potential as promising lead compounds for targeting HtrA1.

Following the molecular docking simulations, the top five compounds with the most favorable binding profiles were identified and reported. These compounds hold significant promise for further exploration and could serve as valuable starting points in the development of novel therapeutic agents targeting HtrA1.

By investigating the binding potential of Syzygium cuminii phytoconstituents with HtrA1 and highlighting the compounds with the highest binding affinity, this study contributes to the understanding of the molecular interactions involved. These results have effects on the building of new drugs aimed at modulating HtrA1 activity and may pave the way for the discovery of effective treatments for conditions associated with HtrA1 dysregulation.

## 2. Methodology
### 2.1 Data collection
A curated compendium of Indian medicinal plants, phytochemicals, and therapeutic applications is the IMPPAT database. Researchers from Chennai, India's Institute of Mathematical Sciences created it (Mohanraj et al., 2018). Over 4,000 Indian medicinal plants, 17,000 compounds, and 1,000 therapeutic applications are all included in the database. In this study, the IMPPAT database was used to collect phytoconstituents of Syzygium cuminii. A total of 241 compounds were collected for the plant. All the compounds were minimized with Open Babel integrated with PyRx using the universal force field and conjugate gradient for 200 steps (Dallakyan et al., 2015; O'Boyle et al., 2011). After that, all the compounds were converted to pdbqt format for molecular docking.

### 2.2 Protein Data Bank
The protein data bank (PDB) is a free online database of 3D structural data for proteins, nucleic acids, and other biological molecules (Burley et al., 2017). The three-dimensional structure of the selected protein HtrA1 (PDB ID: 3TJO, 231 amino acids) was downloaded from the PDB (Eigenbrot et al., 2012). Before docking, the protein was prepared by removing co-crystallized water, adding hydrogen atoms, and optimizing the pka states using the Chimera DockPrep module (Goddard et al., 2005).

### 2.3 Virtual Screening
The virtual screening was a successful first step in the identification of potential drug leads for this protein. Virtual screening was performed using the PyRx autodock vina module (Dallakyan et al., 2015). The blind docking approach was used to cover all important hotspots in the proteins. The grid was generated to cover the complete protein (X,Y,Z coordinates: 46.51, -22.77, 26.62; dimensions X, Y, Z: 63Å, 38Å, 60Å). The grid was generated to cover the complete protein. Molecular docking was then conducted. After the completion of molecular docking, the poses were ranked based on binding energy. The interaction of the top five poses was visualized with the free maestro visualizer. PyRx provides output in pdbqt format, so the poses were converted to pdb format using open babel for visualization.

## 3. Results
After the blind molecular docking, three hotspot regions were found in HtrA1 (fig 1 F). Among the top five compounds, three compounds 3-O-Acetyloleanolic acid (-7.7 kcal/mol), (3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic_acid (-6.6 kcal/mol), and Betulinic_acid (-6.0 kcal/mol) bind in the same region (active site 1). Corilagin (-7.1 kcal/mol) and Friedelonol (-7.4 kcal/mol) are bound to two distinct sites designated as active site 2 and 3 respectively. The location of the three binding sites were shown in figure 1.
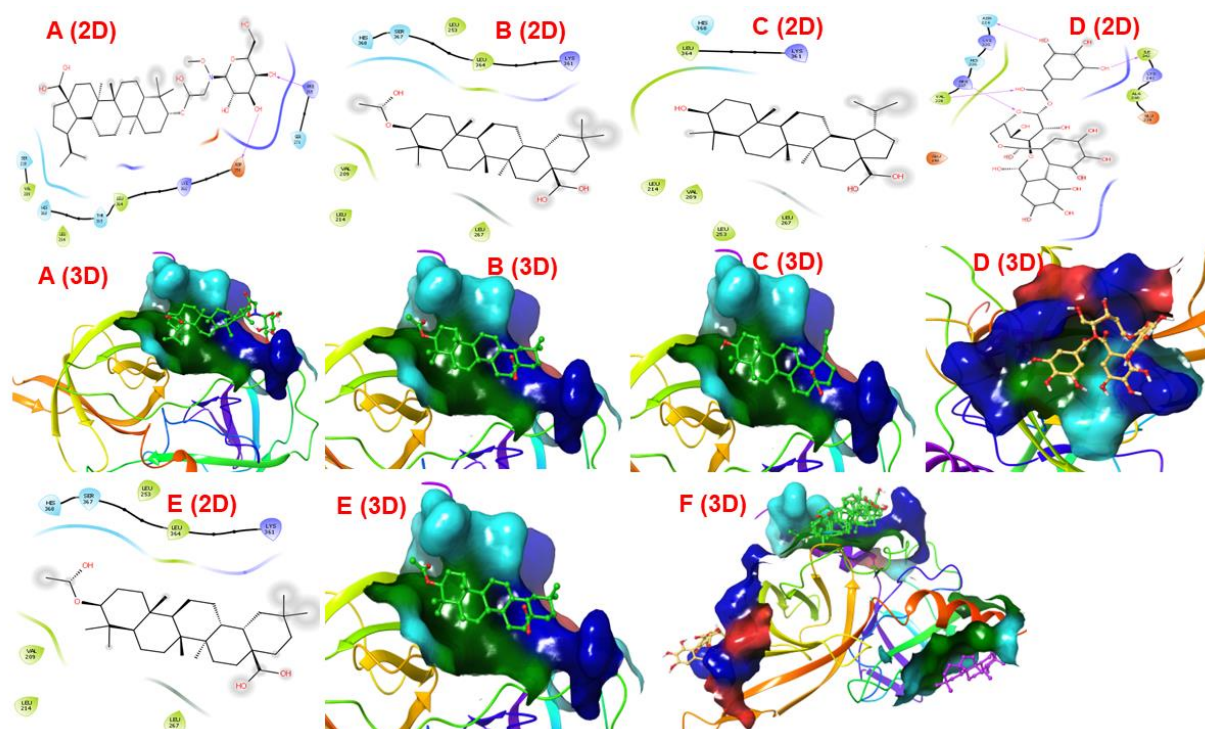


**Figure 1: The blind molecular docking study of molecules with the protein HtrA1. The 2D and 3D structures for the molecules were, A.** (3S)-O-(N-Methoxy-N-D-glucosylglycyl) betulinic acid, **B.** 3-O-Acetyloleanolic acid, **C.** Betulinic acid, **D.** Corilagin, **E.** Friedelon, and **F.** active sites identified active sites in HtrA1.

The three distinct active sites on HtrA1 were identified in this study. The first active site, referred to as activesite1, consists of Val209-Ser210, Leu213, Leu2253, Leu267-Ser271, and Asp358-His368. Active site 2, the second active site, is comprised of Tyr169-Ala173, Val176, Phe278, and Asn290-Thr294. Lastly, activesite3, the third active site, encompasses Glu198, Asn224-Val229, and Glu239-Lys243.

(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic_acid bound to Arg269, Asp358 via hydrogen bonding. 3-O-Acetyloleanolic_acid and Betulinic_acid established themselves in a hydrophobic cavity. Corilagin form hydrogen bonds with Asn224, Ile242, Arg227 and Val228. Friedelon also established themselves in a hydrophobic cavity.

Conservation analysis reveals that via MSA revealed that position 227, 228 and 242 is highly conserved for positively charged and hydrophobic amino acids respectively.

## 4. Discussion

Inflammation and joint destruction are hallmarks of the chronic inflammatory disease rheumatoid arthritis (RA). The search for effective medicinal compounds to manage RA has led to the investigation of various natural products, including (3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid, 3-O-Acetyloleanolic acid, Betulinic acid, Corilagin, and Friedelon (Antonisamy et al., 2011; Oliveira-Costa et al., 2022; Widowati et al., 2022). These compounds have shown promising potential in the treatment and management of RA, as evidenced by several studies.

(3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid, a derivative of betulinic acid, has considerable anti-inflammatory effects, according to reports. According to studies, this substance reduces the production of pro-inflammatory cytokines such interleukin-1 beta, tumor necrosis factor-alpha, and interleukin-6 (IL-6). Additionally, a crucial regulator of inflammation known as nuclear factor-kappa B (NF-B) has been reported to be suppressed by it. All of these processes work together to lessen the inflammatory reactions connected to RA (Choi et. al., 2016).

Another compound, 3-O-Acetyloleanolic acid, has demonstrated anti-arthritic effects in preclinical studies. It exerts its therapeutic potential through the inhibition of enzymes involved in inflammation, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). By suppressing the production of inflammatory mediators like prostaglandins and nitric oxide, 3-O-Acetyloleanolic acid effectively reduces inflammation and provides relief in RA.

Betulinic acid, a naturally occurring triterpenoid, has shown promising immunomodulatory effects in RA. Studies have indicated that betulinic acid inhibits the activity of pro-inflammatory enzymes, including COX-2 and matrix metalloproteinases (MMPs), which are involved in cartilage degradation. Moreover, according to the reports, it suppresses the expression of inflammatory cytokines, such as interleukin-17 (IL-17) and interleukin-6 (IL-6), thereby alleviating the inflammatory response and attenuating joint damage in RA.

Corilagin, a tannin found in various medicinal plants, has exhibited potent anti-inflammatory properties. It has been discovered to reduce the creation of cytokines that are pro-inflammatory, such as TNF-α and IL-1β, in RA models. Additionally, corilagin has been demonstrated to prevent NF-B activation, thus reducing the expression of inflammatory genes. These anti-inflammatory effects make corilagin a potential therapeutic candidate for RA treatment.

Friedelon, a pentacyclic triterpenoid, has also shown promise in RA management. By preventing the generation of pro-inflammatory mediators, it has anti-inflammatory properties, such as prostaglandins and leukotrienes. Furthermore, Friedelon has been reported to exhibit antioxidant effects, which can help alleviate oxidative stress-induced inflammation in RA.

Overall, (3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid, 3-O-Acetyloleanolic acid, Betulinic acid, Corilagin, and Friedelon demonstrate considerable medicinal activity against rheumatoid arthritis. These compounds exhibit anti-inflammatory properties, modulate the immune response, inhibit inflammatory enzymes, and reduce the synthesis of cytokines that are inflammatory. Further research and clinical trials are necessary to validate their efficacy, safety, and potential for therapeutic use in RA patients.

## 5. Conclusion

In conclusion, our comprehensive molecular docking study identified several natural compounds with potential therapeutic benefits in the management of rheumatoid arthritis. The compounds (3S)-O-(N-Methoxy-N-D-glucosylglycyl)betulinic acid, 3-O-Acetyloleanolic acid, Betulinic acid, Corilagin, and Friedelon exhibited strong binding interactions with HtrA1, highlighting their potential as candidates for further investigation. These findings contribute to the expanding repertoire of natural compounds that could potentially be developed into novel therapeutics for rheumatoid arthritis. However, additional studies are needed to elucidate their mechanisms of action, validate their efficacy, and assess their safety profiles before their clinical application.

### Acknowledgement

### Conflict of interest

The authors declare that they have no known competing financial interests.

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