**Investigation of anti-arthritis compounds from *Cinnamomum zeylanicum* through *in-silico* methods**

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**ABSTRACT**

**Arthritis is the inflammation or swelling of one or more joints. It causes joint pain and stiffness and it describes more than 100 conditions that effects the joints, tissues and connective tissues.**

Using molecular docking, the present study investigated the anti-arthritic activity of phytochemicals from *Cinnamomum zeylanicum*. Three different target proteins Dihydroorotate Dehydrogenase, Tyrosine kinase and Bruton’s tyrosine kinase was selected as targets. Virtual screening was done using the tool PyRx.148 phytochemicals were screened against the three selected target proteins. Based on binding energy and hydrogen bond analysis, the top five hit molecules showing activity against all the target proteins were identified, namely 1,3 bis(cinnamoyloxymethyl)adamantine, Ellagic acid, Procyanidin B2, Quercetin and Quercetin-3- O-alpha-l-rhamnopyranoside. The drug-likeness score was calculated for the hits. The hits showing negative druglike properties were eliminated from further ADME and toxicity analysis. The results indicate that among the selected compounds, Quercetin and Quercetin-3-O-alpha-l-rhamnopyranoside showed promising anti-arthritic activity and can be considered as a lead molecule for further in vitro studies.

**Keywords**: *Cinnamomum,* Rheumatoid arthritis (RA), Quercetin, Quercetin-3- O-alpha-l-

rhamnopyranoside, *in-silico.*

**INTRODUCTION**

Arthritis is an age-old disease condition that causes acute or chronic joint inflammation. The disease affects about 0.1– 2.0% of the total world population. The disease was prevalent in Neanderthals and ancient Egyptians (Senthelal *et al.* 2022). The term arthritis was derived from the Greek word "disease of joint". Rheumatoid arthritis (RA) is an inflammatory condition caused by genetic factors. The symptoms of the disease were explained in Charaka Samhita (Sturrocket al., 1977). The term rheumatoid arthritis was coined by Archibald Garrod in the manuscript “Treatise on Rheumatism and rheumatoid arthritis” (Entezami et al. 2011). It is a multifactorial disease; the prevalence rate of which differs according to the area. The etiology of the disease is not clearly understood yet. The T cells, B cells, and the orchestrated interaction of pro-inflammatory cytokines are considered to play key roles in the pathophysiology of rheumatoid arthritis (Pal Singh et al. 2018). Genetics, autoimmunity and environmental factors play a significant role in the onset of RA. Over the last years, several studies revealed that the pathogenesis of RA was driven by a variety of inflammatory cells together with a complex network of cytokines which led to joint destruction, loss of function, and systemic manifestations. The widespread release of cytokines, including tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), plays a crucial role in proinflammatory conditions, thereby losing physiological homeostasis (Smole et al. 2012).

Medicinal plants are an integral part of the traditional treatment system and play a vital role in preventing and treating disease conditions. Use of herbal preparations dates back to prehistoric time and continues to be used by traditional practicians and also serve as household remedies. *Cinnamomum* is one of the most common spices used all over the world (Rao and Gan 2014). It is a well-studied medicinal plant with multiple pharmacological indications. The plant is widely distributed in Southern and North-eastern parts of India. In Ayurveda, it is the main ingredient in several concoctions and medicinal preparations. *C zeylanicum* essential oil contains various phytochemicals such as E-cinnamaldehyde, linalool, beta-caryophyllene, eucalyptol, and eugenol which have remarkable anti-oxidant and anti-microbial properties (Yakhchali et al. 2021). *C. zeylanicum* bark essential oil has been used for thousands of years in Ayurvedic medicine to soothe aching joints and numb pains.

In the present study, in silico analysis of the phytoconstituents of Cinnamomum zeylanicum was done against three proteins **Name of Proteins..Short descrption with reference (probably you can find in discussin)** Three target proteins were selected to evaluate the activity of *C.zeylanicum.* Dihydroorotate Dehydrogenase has a pivotal role in uridine biosynthesis and is also the target for Leflunomide, an approved drug for RA (Leban and Vitt, 2011). It is the rate-limiting enzyme in pyrimidine biosynthesis, which makes it an attractive drug target (Aronson, 2016). Tyrosine kinase JAK3 or Janus kinase is the second target selected for the study. These are involved in signal transduction pathways. Janus kinase three is involved in the critical step leading to the activation and progression of RA (Malemud, 2018). Many JAK inhibitors are currently under clinical trials for development to anti-arthritis therapy (Qiu*et al*., 2019). One of the approaches for treating rheumatoid arthritis is targeting the B cells. The third selected target Bruton kinase is involved in the proliferation of B cells and is a well-approved drug target for arthritis (Lv*et al*., 2018).

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**MATERIALS AND METHOD**

*Cinnamomum zeylanicum* Blume, belonging to the family Lauraceae was selected to study the anti-arthritic activity.

**Preparation of Ligand Molecule**

Information regarding the identified phytochemicals was retrieved using various bioinformatics tools and databases. The molecular weight and Molecular formula of the chemicals were retrieved from the chemical databases Pubchem (Bolton et al. 2008). Compounds that were not available in databases, the structure was drawn using Chemsketch and the details were obtained. The three-dimensional structure of selected phytochemicals in .pdb format were generated using the software tool CORINA (Sadowski et al. 1994).

**Preparation of Target Molecule**

The primary function and sequence of the selected proteins were accessed from the Uniprot database. Using the sequence as input, the physiochemical parameters and the secondary structure composition of the protein were analyzed using an Expasy tool-Protparam and SOPMA tool, respectively (Gasteiger et al. 2005). NCBI gene was accessed to know more about the gene details concerning the selected target proteins. The structure details of the targets and the three-dimensional structure was obtained from Protein data Base (PDB) (Bernstein et al. 1977). The active site residues were predicted both based on tools like CASTp, PDBSum and also upon literature references. Before the virtual screening experiment, the proteins were further prepared using AutoDock 4.2 (Morris, 2009). Water molecules present in the targets were removed and polar hydrogens were added. Non-amino acid residue present in the proteins were removed. The total charge of the protein was calculated and saved in .pdb format.

**Virtual Screening**

Phytochemicals were screened against the selected proteins using the tool PyRx (Duncan 2015). Before the screening, the proteins were prepared using Autodock 4.2. The active site residues of each protein were selected and a grid was set for the docking process. The result was analyzed based on the free energy of binding. Based on the score top molecules were identified and further analysis was done. The interaction of the selected hit molecules with the target proteins was studied to know the mode of binding between ligand and protein. Hydrogen bond, pi, and Van der Waals interaction were observed (Saurabh et al. 2020). Visualization software was employed to analyze the binding mode of ligand inactive site cavity of protein.

**Toxicity Predictions**

ADME and toxicity were analyzed using the web-based prediction tool PreADMET (Lee et al. 2003). The tool can predict ADME, toxicity and drug-likeness properties of chemical molecules. SMILES notation was given as the input.

**RESULTS**

**Preparation of Ligand Molecule**

Through data retrieval from literature and various phytochemical databases, a total of 148 phytochemicals from *Cinnamomum zeylanicum* were identified (Table 1). Out of the total identified chemicals, structural data of 16 chemicals were not present in any chemical databases. The structure was modelled using the tool Chemsketch and SMILES notation was generated. For all the molecules 3D structure in .pdb format was generated using CORINA, with SMILES string as input.

**Selection of Target Protein**

Critical regulators in cell signaling and metabolic pathways in pathogenesis of RA need to be identified as target molecules for treatment. In the present study, three target molecules Dihydroorotate Dehydrogenase, Tyrosine-protein kinase JAK3 and Bruton's tyrosine kinases were selected.

DihydroorotateDehydrogenaseiscodedbythegeneDHODHpresentinchromosome16.The pathway analysis showed that the protein is involved in the UMP biosynthesis *via the* de novo pathway and is located in the mitochondrial innermembrane (Hubackova et al. 2020).Primarysequenceanalysisshowedthat the protein was made up of 395 amino acid residues with a molecular weight of 42867.26 and a pI value of 9.66. Analysis of amino acid composition showed that the protein has a higher percentage ofLeucine(12.7%)followedbyGlycine(11.4%)withanaliphaticindexof97.49andhydropathicity of-0.204. SOPMA analysis done for secondary structure prediction revealed that the protein has an abundance of the alpha helix (43.87%) followed by the random coil (35.42%), extended sheet (12.53%) and beta turns (8.17%). The three-dimensional structure (PDB id: 1d3g) was downloaded from Protein Data Bank. Active site residues were identified from both literature analysis and also fromCASTp.

Tyrosine-proteinkinaseJAK3 islocatedonchromosome19and iscodedbygeneJAK3 (Bergmann et al. 2014).Theprotein hasamajorroleinintracellularsignaltransductionandisexpressedinimmunecells.Theproteinhas 1124 amino acid residues with a molecular weight of 125098.89 and a pI value of6.77. 13,4% of the proteinwascomposedofamino acidleucine.Thealiphaticindexandhydropathicityvaluesare91.64 and -0.148 respectively. Secondary structure prediction revealed that the protein is composed of the alpha helix (41.59%), Random coil (34.86%), extended strand (15.60%) and beta turns (7.95%). The protein 3D structure with PDB id 3lxk was downloaded and active sites were identified. Bruton Tyrosine Kinase, located in chromosome X is coded by gene BTK. The protein plays a crucial role in the development of B cells (Pal Singh et al., 2018). Protparam analysis showed that the total number of amino acids be 659 with a molecular weight of 76281.24. The calculated isoelectric point for the selected proteinis 7.83. The major constituent is identified as glutamic acid followed by leucine, serine and lysine.

**Analysis of Hit molecules**

Analysis of the five top hitmolecules,afterremovingthecommoncompoundsweredone. The toxicity including hepatotoxicity, skin sensitization and mutagenicity was checked using pkCSM toxicity. None of the hit molecules showed any adverse effects. Further molecular propertyanalysisdetailingthehydrogenbonddonoracceptor,logp,PolarSurfaceArea(PSA)andseveralstereoisomerswerecalculated.Toensurethecapabilityofthehitmoleculetoactasapotentialdrug,thedrug- likeness score was also calculated based on the properties of currently marketed drugs and non-drugs. The result showed that among the 5 hit molecules three of them show a negative value, indicating that thepropertiesarelesssimilartotheFDAapproveddrugs.The detailsofthepropertyanalysisaregiven inTable No 4-6

All the common lead molecules showed an acceptable range in the ADMET parameters. Although all compounds showed poor caco2 permeability, they showed good intestinal absorption whichisessentialfororaldrugs.ThecompoundsareeitherPgpsubstratesorinhibitors,whichshould be considered during drug development. Poor VDssvalue should be taken into consideration while adjusting the dosage of the drug compound. All the compounds showed low permeability to the Blood-Brain barrier and Central Nervous System (Table No 7). Metabolic parameters check the association of compound with Cytochrome P450 enzymes, which is essential to study the drug interactions at the later stage of drug development. Procyanidin B2 cannot be considered as it showed a negative clearance rate from the body which can cause toxicaccumulation (Table No 7).

From the docking score and also considering the various analyses like ADME,toxicity prediction and drugs-likeliness analysis done on the hit molecules, QuercetinandQuercetin-3-O-alpha-l-rhamnopyranosidecanbeconsideredaslead moleculesagainst arthritis. The compounds exhibited high binding affinity against three target proteins responsible for the onset of arthritis and also showed a good drug-likeness score and other molecular properties. Finally, the ADMET analysis also showed that these compounds have acceptable properties without any toxic effects and can be considered for further drug development. The bound conformation and the interaction of selected molecules with the three target proteins are given inFigure3-5.

# Comparison with native ligands

The selected three-dimensional structure of Dihydroorotate Dehydrogenase (1d3g) was bound withaninhibitormoleculeBRE.AutoDocktoolwasemployedtodockthenativeligandtothe targetusingthesameparametersusedforvirtualscreening.Theprotein-ligandcomplexformed binding energy of -8.4. Similarly, binding pattern of native ligands present in JAK3 and Bruton’s kinase was also performed and results were found to be -7.8kcal/moland -10.6kcal/mol,respectively.TheselectedstructureofJAK3wasboundtoaninhibitormolecule with ligand id MI 1 and Bruton’s tyrosine Kinase (5p9k) with inhibitor7G8.

**Conclusion**

Thecurrentinvestigationattemptedtoinvestigatethecapabilityofphytochemicalsfromaplant *Cinnamomum zeylanicum* (cinnamon) against three different proteins the Dihydroorotate Dehydrogenase, Tyrosine kinase, Bruton’s tyrosine kinase. Based on virtual screening and molecular docking analysis using various tools and software such as PyRx, AutodockVina, the compounds, namely Quercetin, a flavonoid compound and its derivative Quercetin-3-O-alpha-l- rhamnopyranoside, were identified as promising lead moleculesto against RA. The present *in- silico* study proved that these phytochemicals could be effective for treating arthritis. It is widely accepted that the outcome of an *in-silico* study needs experimental validations. The present *in- silico* studies showed that the plant has the potential to act as an anti- arthritic agent. In order to confirm the results further *in vitro* studies using the isolated target, proteins are recommended. Further studies are required to analyze its potential as the best oral drug through various pre-clinical and clinical trials.