

***In silico* analysis of compounds characterized from *Pseudarthritis viscida* against rheumatoid arthritis**

Bincy Baby, Priyanka James, Ravisankar Valsalan, Sangeetha P. Davis and P.A. Nazeem*

Bioinformatics Centre (DIC), College of Horticulture, Kerala Agricultural University Vellanikkara, Thrissur- 680 656, Kerala, India.

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Abstract

Pseudarthritis viscida is an ethno-medicinal plant and is a component of *Dasamoola* in Ayurveda. The plant is used in the treatment of diseases such as fever, dysentery, cardiac ailments, rheumatoid arthritis and is also effective in the fast healing of fractured bone. Results from multiple research studies emphasize the therapeutic importance of bioactive principles of *Pseudarthritis viscida* in the treatment of rheumatoid arthritis. However, the exact compounds and their mechanism in anti-arthritis action are still to be fully comprehended. Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods to stimulate drug-protein interaction. In the present study, a set of putative targets associated with arthritis such as nitric oxide synthase, janus kinases, cyclooxygenase-2 and prostaglandin E2 were identified. Twenty four compounds identified from *Pseudarthritis viscida* were utilized for subsequent molecular docking simulation studies to elucidate their molecular interactions with the identified targets. The 3D structures of the targets were retrieved from Research Collaboratory of Structural Bioinformatics Protein Data Bank (RCSB PDB) and the structures of phytochemicals were retrieved from NCBI PubChem database. These phytochemicals along with targets were submitted to the CDocker protocol of Discovery Studio 4.0 to perform docking simulations. The molecular interactions were screened based on CDocker energy, CDocker interaction energy, hydrogen bonding and various toxicity parameters. N-Methyltyramine and dalbergioidin had better binding potentials against rheumatoid arthritis that occur due to the over expression of nitric oxide synthase and cyclooxygenase respectively.

Keywords: Rheumatoid arthritis, Docking, Phytochemicals, Discovery Studio 4.0, *Pseudarthritis viscida*

Introduction

Rheumatoid arthritis (RA) is chronic, progressive autoimmune disease characterized by inflammation of joints, destruction of cartilage and bone around the joints, causing deformation of the joints. The joint inflammation of rheumatoid arthritis causes swelling, pain, stiffness, and redness in the joints (Koch, 1998). Rheumatoid arthritis is initiated by lymphocytes that localize at synovium, a membrane that surrounds joint where they get activated causing pain and swelling. These lymphocytes produce protein mediators (cytokines) of inflammation, attract other immune cells to the site, activate resident cells and cause uncontrolled synovial fluid production (Choy, 2012). A balance between pro-

inflammatory and anti-inflammatory cytokines is necessary for the development of a regulated immune response. Excess production of pro-inflammatory cytokines or the deficiency of anti-inflammatory cytokines may lead to immune pathology. The over production of the pro-inflammatory cytokines such as tumor necrosis factor α (TNF- α), interferon- α (IFN- α), interleukin-6 (IL-6) and IL-17 can promote autoimmune pathology (Shivaprasad et al., 2014).

Genomic and proteomic strategies are being used to identify potential molecular targets. The activities of pro-inflammatory cytokines are positively regulated by certain molecules involved in cytokine signaling. These include nitric oxide synthase

*Author for correspondences: Phone-0487- 2371994 ; E-mail: bic@kau.in

(NOS), janus kinases, cyclooxygenase and prostaglandin E2.

In inflammation, inflammatory cytokines induce the expression of inducible nitric oxide synthase (iNOS), which produces high amount of nitric oxide (NO) for prolonged period. NO has regulatory and pro-inflammatory properties in inflammation. The increased expression of iNOS in rheumatoid arthritis and NO production have been shown to have detrimental effects (Van't Hof et al., 2000). Janus kinases include JAK1, JAK2, JAK3 and TYK2 belonging to the family of non-receptor tyrosine kinase. These play important roles in the cytokine-mediated Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signaling pathway which is critical for the survival, proliferation, development and differentiation of a variety of cells involved in the immune system. Interestingly, mutation or increased localized concentration of JAK3 or gamma chain causes over activation of JAK-STAT signaling, which results in various inflammatory diseases (Kubler, 2014). Cyclooxygenase-2 (COX-2) is the key enzyme involved in the biosynthesis of prostaglandins, which are responsible for mediation of inflammation, pain and increased body temperature. COX-2 enzyme is involved in characteristic responses of pain in rheumatoid arthritis. When the activity of cox-2 is blocked, inflammation is reduced with no side effects at gastric and renal level (Stańczyk and Kowalski, 2001; Kvien et al., 2015). Prostaglandin E2 (PGE2) is a key mediator in inflammatory response and has emerged as an interesting drug target for the treatment of pain. Prostaglandins are lipid inflammatory mediators and play both beneficial and harmful roles during inflammation according to their site of action and the etiology of the inflammatory response. With respect to the role of PGs in inflammation, they can be effective mediators in the pathophysiology of RA (Mc Coy et al., 2012).

The modulation of immune response by using medicinal plants and its secondary metabolites as

possible therapeutic measures has become a subject of active scientific investigation. It is reported that the phytoconstituents like flavonoids, terpenoids, saponins have anti-inflammatory activity (Agnihotri et al., 2010). The therapeutic potentiality of *P. viscida* on arthritis has been reported by *in vitro* and hence it is possible that the active constituents in *P. viscida* may be involved in the active inhibition of the inflammatory mediators (Subramoniam et al., 2013). The present study is focused on the therapeutic potential of *Pseudarthria viscida* in rheumatoid arthritis using computational methods towards the discovery of new chemical entities as drug leads. Computational techniques can strongly support designing novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction (Tiwari et al., 2012). Molecular docking approaches are routinely used in modern drug design to identify small molecules by orienting and scoring them in the active binding site of a protein.

Materials and Methods

Protein identification and preparation

The reported molecular targets responsible for rheumatoid arthritis such as nitric oxide synthase, janus kinases, cyclooxygenase-2 and prostaglandin E2 were selected for the interaction study (Stańczyk and Kowalski, 2001; Koo et al., 2012; Kubler, 2014;).

The X-ray crystallographic structures of these target proteins were retrieved from protein data bank (PDB). The retrieved PDB structures contain water molecules, heavy atoms, cofactors, metal ions etc. and these structures do not have information about topologies, bond orders and formal atomic charges. Hence the downloaded PDB structures were prepared using 'prepare protein' protocol of Discovery Studio 4.0. The target proteins were prepared by removing all water molecules, ligands and other hetero atoms from the structures. Hydrogen atoms were added to the atoms to satisfy their valencies. The structures were then energy minimized by applying CHARMM force field to

remove the steric clashes between the atoms in order to get stable conformation.

Active site identification

The binding sites of the receptor proteins were predicted based on 'receptor cavity method' using Accelry's Discovery Studio 4.0. Using this protocol, active sites of the target receptor were identified based upon the inhibitory property of the amino acid residues present in the binding sites.

Ligand preparation and filtration

A collection of 24 phytochemicals from *Pseudarthria viscida* were taken as ligands for docking analysis. The 3D structures of these compounds were downloaded from PubChem database. These ligands were then cleaned up, calculated 3D coordinates and generated ligand conformations by applying 'prepare ligand protocol' of Discovery Studio 4.0. After preparation, the compounds were filtered based on the molecular properties for predicting their solubility and permeability in drug discovery. The best known of the physical property filters is Lipinski's "rule-of-five", which focuses on bioavailability. The rule states that the compounds have molecular mass less than 500 daltons, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octanol-water partition coefficient log P not greater than 5 (Lipinski et al., 2001). The filtered compounds were then used for docking analysis.

Docking

The anti-inflammatory activity of all the 24 phytochemicals reported from *P. viscida* was assessed by docking these compounds against the respective active sites of the target proteins. Discovery studio 4.0 was used in this study to find the interacting compounds of *P. viscida* with the selected targets of arthritis. Strategies of Discovery Studio 4.0 are to exhaustively dock or score possible positions of each ligand in the binding site of the proteins. Docking study of the target proteins was done with natural compounds derived from *P. viscida* to find the preferred orientation and binding

affinity of the compounds with each target protein using scoring functions. A molecular dynamics (MD) simulated-annealing-based algorithm, namely, CDOCKER was used to score the interacting compounds. This method uses a grid-based representation of the protein-ligand potential interactions to calculate the binding affinity (Wu et al., 2003). CDOCKER uses soft-core potentials, which are found to be effective in the generation of several random conformations of small organics and macromolecules inside the active site of the target protein. Ligands were docked to the proteins followed by scoring them for their relative strength of interaction to identify candidates for drug development. The final poses were then scored based on the total docking energy, which is composed of intramolecular energy of ligand and the ligand-protein interaction. The lowest energy structure was taken as the best fit. Interpretation of the values was done using standards provided by Discovery Studio such as CDOCKER energy, CDOCKER interaction energy, hydrogen bonds, binding energy etc.

Drug likeliness

Drug-likeness is a qualitative concept used in drug design to evaluate how the substance acts like drug with respect to factors like bioavailability. The molecular properties which influence absorption, distribution, metabolism, excretion and toxicity are recognized as a long side therapeutic potency as key determinants of whether a molecule can be successfully developed as a drug (Zhang et al., 2012). These parameters are responsible for about 60 percent failures of all drugs in the clinical phases and so the prediction of ADME/T properties plays a significant role in new drug discovery process (Hire et al., 2012). Thus, it has become imperative to design lead compounds which would be easily orally absorbed, easily transported to their targeted site of action, not easily converted into toxic metabolic products and easily eliminated from the body before accumulating in sufficient amounts. The ADMET properties of the compounds were analyzed for drug like candidates.

Results and Discussion

Protein preparation and active site identification

The three dimensional structures of the identified target proteins, nitric oxide synthase, janus kinases, cyclooxygenase-2 and prostaglandin E2 were retrieved from the protein data bank. The PDB ID for nitric oxide synthase, janus kinases, cyclooxygenase-2 and prostaglandin E2 are 3E7G, 4HVD, 3LN1 and 4AL0 respectively. The energy minimized downloaded protein structures are given

in Figure 1.

The active sites along with the amino acid residues having inhibitory properties of each target protein are given in Table 1.

Ligand preparation

Ninety four phytochemicals were generated after ligand preparation process and out of them, 91 satisfied Lipinski rule and are expected to be active compounds after oral administration.

Table 1. Amino acid residues identified in the active sites of targets

Targets	Active site	Active site residues
Nitric oxide synthase(3E7G)	Site 3	Ser118, Phe476, Ile462, Val465, Trp465
Cyclooxygenase-2(3LN1)	Site 7	His75, Arg106, Gln178, Val335, Leu338, Ser339, Tyr371, Arg499
Janus kinases(4HVD)	Site 2	Leu905, Leu956, Glu903, Ala853, Leu828, Lys855, 3E7G
Prostaglandin E2(4AL0)	Site 1	Glu77, Arg73, Asn74, Tyr117, His113, Arg126, Ser127

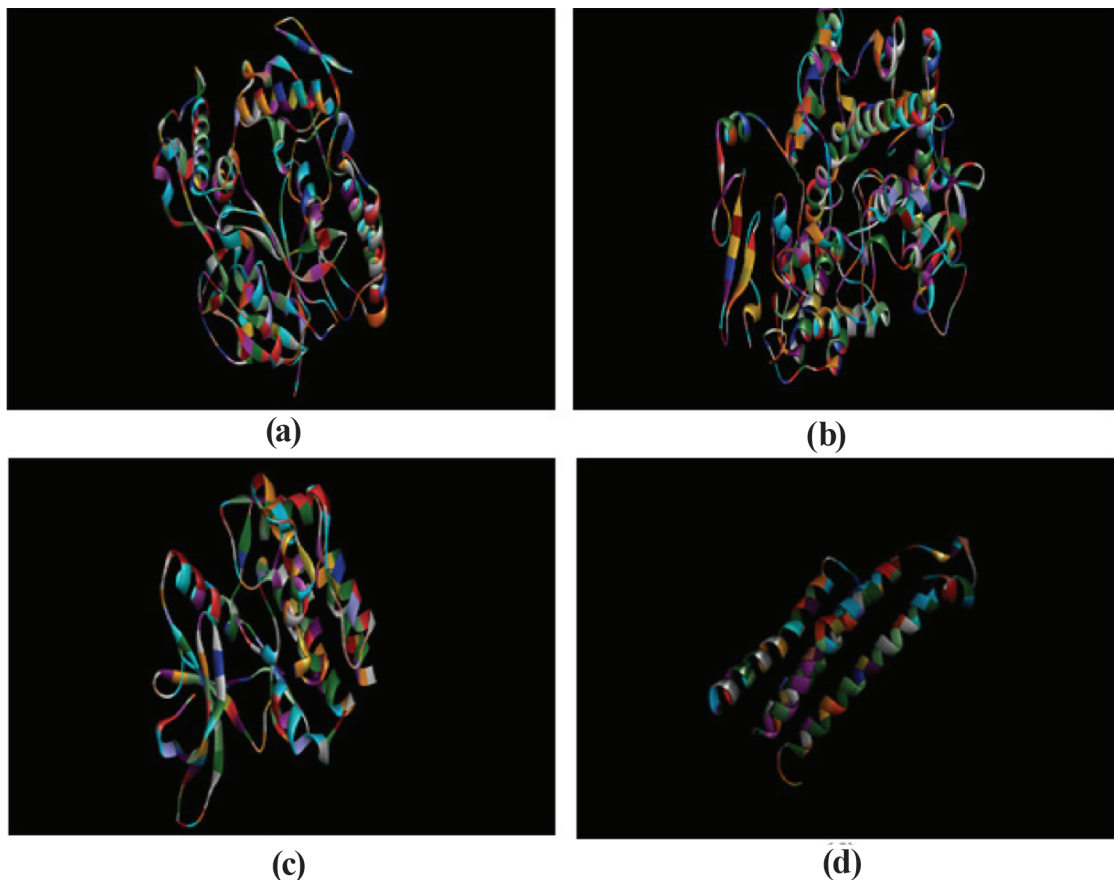


Figure 1. Energy minimized structures of targets. a) Nitric acid oxide synthase (3E7G) b) Cyclooxygenase (3LN1) c) Janus kinases (4HVD) d) Prostaglandin E2 (4AL0)

Table 2. Dock results of phytochemicals with anti-rheumatoid arthritic targets

Target	Ligand	(-)CDOCKER energy	(-) CDOCKER interaction energy	H bond amino acid residues	H bond distance	Binding energy (kcal mol ⁻¹)
Nitric oxide synthase(3E7G)	Quercetin	46.26	49.09	TYR347 TYR373	1.82 1.96	-256.57
	N-methyltyramine	37.49	35.24	ILE 462 VAL465	1.90 2.04	-124.64
	Candicine	19.83	29.68	TYR347 TYR373	2.16 2.43	-109.28
Cyclooxygenase -2(3LN1)	Dalbergoidin	56.6252	60.20	TYR371 ARG499 LEU338	1.83 2.19 2.00	-160.10
	Leucopelargonidin	37.86	53.64	ARG499 LEU338	1.86 1.95	-99.64
	Kievitone	17.87	59.42	ARG499	1.83	-81.52
				SER516	2.28	
				SER516	1.85	
Diphysolone	7.50	44.80	LEU338 SER516 LEU338	1.96 2.07 1.93	-102.18	
Janus kinases (4HVD)	Caffeic acid	29.22	30.87	LEU905	2.06	-52.76
				LEU905	2.36	

Molecular docking

Except prostaglandin E2, all the other three therapeutic targets showed interactions with the phytochemicals. Table 2 describes the better interaction scores of phytochemicals with the targets, nitric oxide synthase, cyclooxygenase and janus kinases.

The ligand molecules with least binding energy are considered as compounds with highest binding affinity. Quercetin, dalbergoidin, N-methyltyramine, candicine and diphysolone were the compounds with least binding energy and the values were -256.57 kcal mol⁻¹, -160.10 kcal mol⁻¹, -124.64 kcal mol⁻¹, -109.28 kcal mol⁻¹ and -102.18 kcal mol⁻¹ respectively. This binding affinity indicated a focused interaction between the above compounds with the targets compared to others. The parameters for finding the best inhibitors such as CDOCKER energy, CDOCKER interaction energy and number of hydrogen bonds were also evaluated. CDOCKER energy is the combined energy produced by the sum of internal ligand strain energy

and receptor-ligand interaction energy where, CDOCKER interaction energy is the interaction energy between the protein and ligand and the values of these two parameters indicate the strength of interaction between the proteins and the ligands. Besides least binding energy, compounds with least atomic energy difference between CDOCKER energy and CDOCKER interaction energy were analyzed. Based on CDOCKER energy and CDOCKER interaction energy, dalbergoidin, quercetin, N-methyltyramine and caffeic acid had favorable interactions. Comparative analysis of receptor ligand interactions depicted the core inhibiting residues on arthritis.

The lead amino acid residues were ILE462, Val465, TYR347, TYR373, LEU338 and ARG499 and LEU905. Quercetin is interacting with amino acid residues, TYR347 and TYR373 of nitric oxide synthase with bond length less than 2.0 Å. The amino acid residues of N-methyltyramine involved in forming the hydrogen bond were ILE 462 and VAL465 with bond length 1.90 Å and 2.04 Å

Table 3. ADME/T evaluation of screened compounds

Ligand	Admet solubility level (2-4)	BbB level (2-4)	Hepatotoxicity (false)	Absorption level(0-1)	Alogp98 (<4)	CYP2D6 (false)
N-methyltyramine	4	2	false	0	1.446	false
Dalbergoidin	3	4	false	0	2.014	false
Quercetin	3	4	true	1	1.63	false
Caffeic acid	4	3	false	0	1.443	false

respectively. The molecular interaction between dalbergoidin and the protein, cyclooxygenase-2 showed strong hydrogen bonds and the residues were TYR371, ARG499 and LEU338 with bond length 1.83 Å, 2.19 Å and 2.00 Å respectively. There were two hydrogen bonds between caffeic acid and janus kinases and the amino acid residue involved in the interaction was LEU905.

From the analysis based upon the strength of interaction, quercetin, N-methyltyramine had good interaction with nitric oxide synthase and dalbergoidin showed better interaction with cyclooxygenase. Caffeic acid showed good CDOCKER energy and CDOCKER interaction

energy scores with janus kinases but had only poor binding affinity compared to other target-ligand interaction. Figure 2 illustrates the hydrogen bond interactions between docked complexes.

ADMET Evaluation

Considering the comparable CDOCKER energy, interaction energy and binding energy, three compounds were forwarded for ADMET analysis. These studies are based on the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the compounds. These properties provide insights in to the pharmacokinetic properties of the compounds and were checked using Discovery Studio's built in ADMET protocol. The various parameters tested in this study were aqueous solubility, Blood Brain Barrier (BBB) level, Hepatotoxicity, Absorption level, AlogP and CYPD26. Pharmacokinetic properties of the best fit phytochemicals showed that two of the compounds had passed all the pharamacokinetic parameters. The compounds that passed the parameters were N-methyltyramine and dalbergoidin. These compounds were thus selected as the best compounds in this study as they had good interaction scores along with ADMET properties. The other compound, Quercetin, showed a hepatotoxic nature and this makes the compound undesirable as a drug candidate.

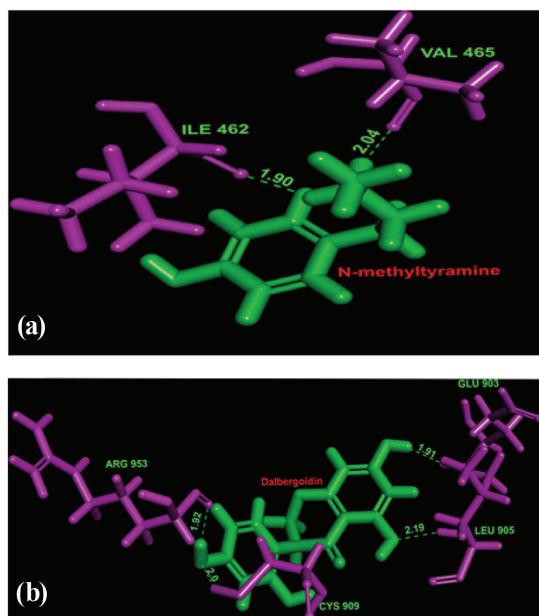


Figure 2. Interaction of compounds with targets (a) Interaction of N-methyltyramine with nitric oxide synthase (b) Interaction of dalbergoidin with cyclooxygenase

Rheumatoid arthritis is a complex phenomenon involving multiple gene action. Four important target proteins were identified in the study and their interactions were studied with 24 phytocompounds in *P. viscida*. The analysis also showed that only 3 of the phytocompounds showed better interaction with the targets, nitric oxide synthase and

cyclooxygenase. The phytochemicals showed poor interaction with janus kinases and prostaglandin. Out of these compounds, Quercetin and N-methyltyramine showed good interaction with nitric oxide synthase while dalbergioidin had good interaction with cyclooxygenase. ADMET studies showed that two compounds, namely dalbergioidin and N-methyltyramine passed all the pharmacological parameters. The other compound, quercetin showed hepatotoxic nature in our studies. From the present study, we conclude that dalbergioidin and N-methyltyramine from *P. viscida* could be the lead compounds to design drugs against rheumatoid arthritis. These results also suggest that these compounds could be used for experimental testing against nitric oxide synthase and cyclooxygenase.

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