*In silico*Analysis and Identification of *Anisomeles* inhibitors against Rheumatoid Arthritis

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ABSTRACT:

Rheumatoid Arthritis is an autoimmune disease which cause chronic inflammation to the joints and other areas of the body. It occurs when the immune system mistakes the body's healthy tissues as foreign invaders and the immune system responds to inflammation which occurs in the target tissue or organ. Drug against the disease is available and are not much effective. So, it is necessary to speed up the researches on developing new and more effective anti-rheumatic drugs. At present, various researches are in progress in evaluating medicinal plants against these types of diseases. *Anisomeles malabarica* is a traditionally used important medicinal plant and the herb is reported to possess anti-spasmodic, anti-periodic properties and is used in treatment of various diseases. The 34 compounds from *Anisomeles malabarica* were identified through proper literature survey and the Human C-Reactive Protein (CRP Protein-1B09) which is the target protein for Rheumatoid Arthritis was retrieved from protein databank. Further Molecular docking was performed using Argus lab for the 34 compounds against the target protein. Later the best docking interactions were compared to the docking interactions of target with the synthetic compounds JAK inhibitors- (Janus kinase inhibitors).

KEYWORD:

Anisomeles malabarica, Anti-rheumatic, Phytochemical, Docking, Synthetic compounds.

INTRODUCTION:

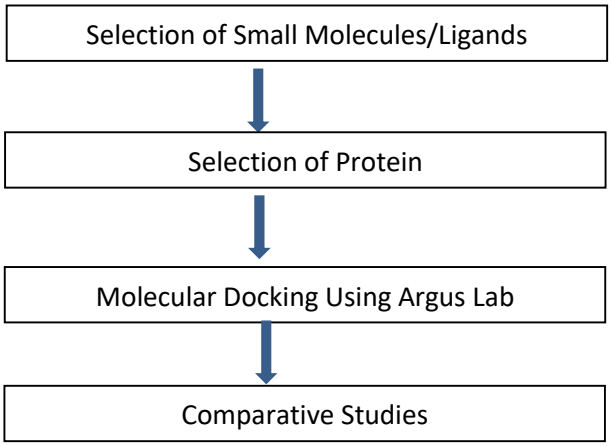
Anisomeles malabarica is a highly aromatic plant and belongs to the family Lamiaceae (Mint family). *Anisomeles malabarica* is distributed in major parts of the South India as a traditional medicinal plant commonly known as Peyviratti (Tamil), Chodhara (Marathi), Karithumbi (Kannada), Perumtumpa (Malayalam). The plant is traditionally used for the treatment of Snakebites as antitode. The leaves of the plant is used in the treatment of rheumatism, carminative, stomachic and the roots of the plant are used as a potential source of drug for ailments such as Asthma, Malaria, Rheumatism, Tuberculosis, Ulcers and Wounds. (Isamil Shareef.M, Leelavathi, 2011) The pharmacological activity of the *Anisomeles malabarica* species is identified to cure the Rheumatoid Arthritis disease. Rheumatoid Arthritis is a chronic inflammatory disorder that can affect more than just your joints. The condition can damage a wide variety of body systems, including the skin, eyes, lungs, heart and blood vessels. An autoimmune disorder, Rheumatic Arthritis occurs when your immune system mistakenly attacks your own body's tissues.

The 34 compounds of *Anisomeles malabarica* were identified through PubMed, a database for biomedical literature and journals. Human C - reactive protein (CRP Protein) is one of the protein which causes the disease Rheumatoid Arthritis. Rheumatoid arthritis (RA) is an autoimmune condition that causes the body to attack healthy tissues in joints. This triggers inflammation, which causes joint pain, swelling, and stiffness. Blood tests can help confirm a diagnosis of RA. People with the disease often have elevated levels of certain proteins, including C-reactive protein (CRP), in their blood. The liver makes CRP, a type of protein. When the immune system signals inflammation, the body sends CRP through the bloodstream to the affected area. High levels of CRP in the blood indicate chronic inflammation. This occurs with a wide range of health problems, such as infection, obesity autoimmune conditions, including RA cancer and heart disease.

Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months.

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins. It is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified. The structure of the C-reactive protein (CRP) was retrieved from PDB database and Molecular docking was performed between the protein and 35 compounds from *Anisomeles malabarica* to identify the best interactions.

MATERIALS AND METHODS:



Selection of Small Molecules:

The 34 phytochemical compounds were retrieved from biomedical literature survey using PubMed database. PubMed is a free resource developed and maintained by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM). It is free search engine accessing primarily the MEDLINE database of references and extracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval. For Computational analysis, the compound should possess at least 2 dimensional structures. Using the PubChem database the 2dimensional structures of 34 compounds were retrieved. PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely retrieved via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PubChem database.

Selection of Protein:

Human C - reactive protein (CRP Protein) is one of the protein which causes the disease Rheumatoid Arthritis. People with the disease often have elevated levels of certain proteins, including C-reactive protein (CRP), in their blood. The liver makes CRP, a type of protein. When the immune system signals inflammation, the body sends CRP through the bloodstream to the affected area. High levels of CRP in the blood indicate chronic inflammation. This occurs with a wide range of health problems, such as infection, obesity autoimmune conditions, including RA cancer, heart disease. C-reactive protein was the first pattern recognition receptor (PRR) to be identified. The PDB ID for the C-reactive protein is 1B09. The structure of the protein was retrieved from PDB database. (https://www.rcsb.org/)The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy. The PDB is a key in areas of structural biology, such as structural genomics.

Molecular Docking using Argus Lab:

After the selection of the protein and small molecules, molecular docking was performed to see the binding interactions. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominating binding mode(s) of a ligand with a protein of known three-dimensional structure. Argus lab protocol for docking was carried to find the best interactions between the protein and the ligand. Argus lab is a free molecular package that runs under windows. It is installed on all public computers and can be retrieved from www.arguslab.com/downloads.htm/ . It is a molecular modelling, graphics, and drug design program. The Argus lab docking engine, implemented in it, approximates an exhaustive search method with similarities to Dock and Glide. Flexible ligand docking is possible with Argus lab, where the ligand is described as a torsion tree and grids are constructed that overlay the binding site. Ligand's root node is placed on a search point in the binding site and a set of diverse and energetically favourable rotations is created. The 34 phytochemical compounds of *Anisomeles malabarica* and the C - reactive protein of PDB ID 1B09 was used for Molecular docking using the Argus Lab.

Comparative Studies:

The best binding interaction of the phytochemical compounds from *Anisomeles malabarica* were compared to the interactions of synthetic compounds such as JAK for the disease Rheumatoid Arthritis. Janus kinase (JAK) inhibitors, have been proposed as "an encouraging next-generation treatment tool" for rheumatoid arthritis (RA) that could meaningfully contribute to the therapeutic landscape. JAKs are intracellular enzymes that transmit signals from cytokines binding to receptors on the cell surface to signal transducers and activators of transcription (STATs), which drive pro-inflammatory cellular responses. Although treatment with biologic agents results in disease suppression for many patients with RA, only approximately 30% achieve complete remission, and the majority of patients treated with biologics experience disease exacerbation following cessation of treatment. The Synthetic compounds JAK inhibitors were further used for Molecular docking using Argus lab with the same CRP protein. And the interaction was compared with the best docking interaction of phytochemical compounds. The results obtained from the present study later strongly recommends that the phytochemical compounds of *Anisomeles malabarica* leads to the discovery and development of potential drugs for bacterial disease such as Rheumatoid Arthritis.

RESULTS AND DISCUSSIONS:

Preparation of small molecules / ligands:

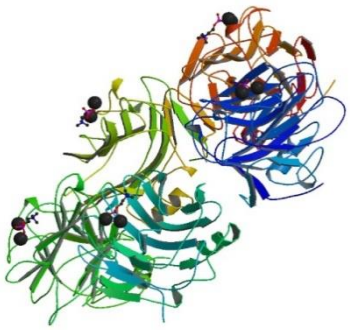
The 34 phytochemical compounds through literature were identified from the plant *Anisomeles malabarica* with the help of PubMed database.

Table 1: Phytochemical Compounds from Anisomeles malabarica

S.No	Compounds
1.	Alpha pinene
2.	Camphene
3.	Beta pinene
4.	3-Octanol
5.	1,8-cineole
6.	Cis-sabinene hydrate
7.	Linalool
8.	Camphor
9.	Borneol
10.	Myrtenol
11.	Alpha-thujone
12.	Linalyl acetate
13.	Nerol
14.	Geraniol
15.	Geranial
16.	Thymol
17.	Bornyl acetate
18.	Terpenyl acetate
19.	Anisole
20.	2-Isopropylbenzaldehyde
21.	Eugenol
22.	N-nonanyl acetate
23.	Delta-cadinene
24.	Isocaryophyllene
25.	Caryophyllene oxide
26.	Epiglobulol
27.	Globulol
28.	Nerolidyl acetate
29.	Farnesyl acetate
30.	Alpha bisabolol
31.	Trans-phytol
32.	Citronellol
33.	Isomenthol
34.	Azulene

Preparation of proteins:

The structure of the C - reactive protein (CRP protein) was retrieved from PDB database of PDB ID 1B09. The three dimensional structure of the protein were retrieved using Protein Data Bank (PDB). The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids.



3D structure of C - reactive protein (CRP protein)
1B09

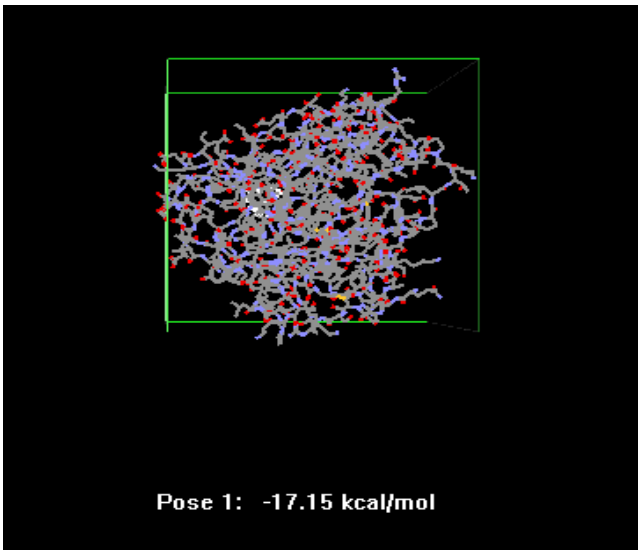
Docking Interactions:

Molecular Docking was performed using Argus lab protocol to find the interactions between the C - reactive protein with the PDB ID of 1B09. The best binding interactions for the protein 1B09 is -17.15 Kcal/mol for the compound Delta Cadinene.

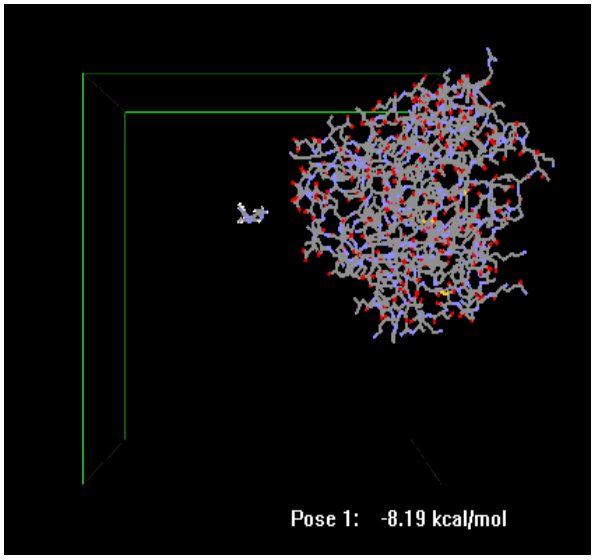
Table 2: Docking Interactions of the ligands and protein.

S.No	Phytochemical Compounds	1B09 Kcal/mol
1.	Alpha pinene	-11.96
2.	Camphene	--7.38
3.	Beta pinene	-11.02
4.	3-Octanol	-8.90
5.	1,8-cineole	-11.39
6.	Cis-sabinene hydrate	-12.03
7.	Linalool	-10.4
8.	Camphor	-10.01
9.	Borneol	-10.29
10.	Myrtenol	-11.63
11.	Alpha-thujone	-7.22
12.	Linalyl acetate	-6.98
13.	Nerol	-10.45
14.	Geraniol	-7.43
15.	Geranial	-6.88
16.	Thymol	-7.54
17.	Bornyl acetate	-9.99
18.	Terpenyl acetate	-10.94
19.	Anisole	-6.73
20.	2-Isopropylbenzaldehyde	-7.72
21.	Eugenol	-11.04
22.	N-nonanyl acetate	-6.99
23.	Delta-cadinene	**-17.15**
24.	Isocaryophyllene	-16.35
25.	Caryophyllene oxide	-15.07
26.	Epiglobulol	-10.19
27.	Globulol	-11.42
28.	Nerolidyl acetate	-14.26
29.	Farnesyl acetate	-12.51
30.	Alpha bisabolol	-10.21
31.	Trans-phytol	-8.32
32.	Citronellol	-7.90
33.	Isomenthol	-9.08
34.	Azulene	-13.19

Best binding energies:



1B09 for Delta Cadinine



1B09 for JAK inhibitors

Comparative Studies:

The synthetic compound Janus kinase –JAK inhibitors were identified for the disease Rheumatoid Arthritis through literature survey using PubMed. The same C - reactive protein (CRP protein) of PDB ID 1B09 was carried out for the Molecular docking protocol to predict the interactions and compare the interactions between synthetic compounds and Phytochemical compounds. The synthetic compound JAK was docked with the same protein -C - reactive protein .Thus the interactions of synthetic compounds were identified to be lesser than the phytochemical compounds.

Table 3:

Comparative Studies: Docking interactions of synthetic compounds and phytochemical compounds.

S.No	Proteins	Phytochemical compounds	Ligand Pose Kcal/mol
1	1B09	DELTA CADINENE	-17.15

S.No	Proteins	Synthetic compounds	Ligand Pose Kcal/mol
1	1B09	JAK inhibitors	-8.19

CONCLUSION:

Docking studies were performed to prove that the phytochemical compounds has the best interactions than the synthetic compounds. By comparing the both docking interactions of phytochemical compounds and synthetic compounds, we identified that the phytochemical compound Delta-cadinene targeting 1B09 has more effect to cure the disease Rheumatoid Arthritis. Thus we strongly recommend that the phytochemical compounds of *Anisomeles malabarica* lead to the discovery and development of potential drugs for bacterial disease such as Rheumatoid Arthritis. Although the synthetic compounds are identified to be the powerful anti-Rheumatoid drugs, it is reported to cause some serious side effects. Hence we recommended that the natural compounds as best inhibitors against Rheumatoid Arthritis. Further *in vitro* studies on natural compounds of *Anisomeles malabarica* could lead to discovery of novel potential drugs against Rheumatoid Arthritis disease.

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