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# A CURATIVE APPROACH FOR PSORIASIS VULGARIS

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## ABSTRACT

Psoriasis vulgaris is a chronic, inflammatory disorder with multiple pathways. It is a skin mediated diseases due to the epidermal keratinoctyes, dermal vascular cells which includes activated antigen presenting cells. Interaction with the activated T cells with monocytes with Th17/IL-23 causes chronic inflammation. IL-17A induces expression of the keratinocyte chemokine CCR6. Trafficking of activated Th17 cells, but not of Th1 or Th2 cells, to psoriatic skin has been associated with CCR6 expression. Structure of the protein CCR6 was modeled using Modeller 9.17. Phytochemical compounds, beneficial effects on the plant host exhibit a series of biological properties that influence the human in a health-promoting manner. Due to their natural origin and low side effects it eliminates the causes and effects of psoriasis. Molecular docking studies were undergone using Auto Dock 4.2 and Docking Server.

**KEYWORDS:** *APCs, IL-17A, CCR6, Th17 cells, Homology Modeling, Molecular Docking, Auto Dock, Docking Server, Modeller 9.17*

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# INTRODUCTION

Psoriasis vulgaris is a genetic, systemic, inflammatory, chronic disorder, which can be altered by environmental factors. It is characterized by disfiguring, scaling, and erythematous plaques that may be painful. Approximately 80% of those affected with psoriasis have mild to moderate disease, with 20% having moderate to severe psoriasis.[1] The disease is mainly due to T-cell mediated autoimmune diseases where the number of those affected is rising. As the psoriatic immune response pattern relates to activated Th-1 cells, psoriasis appear to be mutually exclusive due to the Th-1/Th-2 dichotomy.[2] Psoriasis is a disease in which T lymphocytes are thought to play a vital pathogenic role. The activated memory skin-homing T cells which induce characteristic disease features via cytokine production. Understanding the mechanisms of T cell accumulation in skin and mediation of psoriasis is critical to designing immunotherapy for this distressing disease.[3] Psoriasis is characterized by hyper proliferation and aberrant differentiation of keratinoctyes, dilated, hyper plastic blood vessels as well as an inflammatory infiltration of leukocytes, predominantly into the dermis.[4] It is widely accepted that T helper (Th)1 andTh17 lymphocytes contribute to the disease pathogenesis through the release of inflammatory cytokines that promote further recruitment of immune cells, keratinocyte proliferation, and sustained chronic inflammation.[5] IL-17 (interleukin-17), a hallmark cytokine of Th17 (T-helper 17) cells plays critical roles in host defense against bacterial and fungal infections, as well as in the pathogenesis of autoimmune diseases.[6] Considerations of redundancy have led to the proposal that drugs should be developed targeting combinations of receptors[7] Psoriasis vulgaris is a chronic inflammatory skin disorder, with a worldwide distribution and an increasing incidence and prevalence. Recent epidemiological studies show its impact on a level of 1.5-4%, with important local variations depending on ethnicity and climate.[8] C Chemokine Receptor type 6 (CCR6) was initially identified on dendritic cells and T cells, and was found to be expressed on B cells and subsets of effector/memory T cells from peripheral blood, were able to express IL-17A cells that produce Th17 cytokines.[9] Generally chronic skin diseases typically aren't curable, but they can be managed using drugs. But so many medicinal plants are also used for treating skin diseases.[10] The prescribed synthetic drugs for the treatment of psoriasis are associated with severe side effects; thus, researchers around the globe are searching for new, effective, and safer drugs from natural resources. Virtually all cultures worldwide have relied historically, or continue to rely on medicinal plants for Skin diseases.[11]. In the present study various phytochemical compounds are used in docking studies to compare with the commercial drugs. The herbal medicine is easily available and easy to use in treatment. They play a very important role in management of the skin and inflammatory diseases. [12]

# MATERIALS AND METHODS

### *Target Sequence Retrieval*

The target protein sequence C-Chemokine Receptor type 6 has no modeled structure the sequence was retrieved from UNIPROT accession number P51684 (CCR6_HUMAN). FASTA format of the sequence was obtained. It contains a large amount of information about the biological function of proteins derived from the research literature

### *Protein Sequence analysis*

The target sequence of C-Chemokine Receptor 6 was analyzed with various protein structure analysis tools to find out their amino acid sequence, physiochemical properties, GRAVY (Grand Average of hydopathicity). Secondary structure analysis has done to understand their polypeptide chain and the alignment of sequence families to know their alpha helix, beta-sheet and coil.

### *Homology Modeling*

The target sequence as PIR format was given as input for the program Modeller 9.17 [13,] which generates five best models in a three-step process (a) Template search (b) Target template alignment (c) Model building. After model evaluation based on the DOPE (Discrete Optimized Potential Energy) score the fourth model was chosen as the best model.[14] The models were further energy minimized and visualized using Weblab viewer. The template was selected on the basis of similarity search by BlastP which search against non redundant database with the target sequence.[15]

### *Model Verification and refinement*.

The modeled protein of C-Chemokine Receptor type 6 was modeled and the structure was verified using PROCHECK available in SAVS (Structure Analysis and Verification server),[16] about their stereo chemical quality, geometry of the residues in the given protein structure. Distortions due to ligand binding in the protein's active site are also verified. Model refinement also done to check the quality of the modeled structure. Active site prediction, which helps to find the pockets on the surface of proteins that, may act as a binding site for small molecule ligands.

### *Structure-Structure Alignment*

Structure alignment has been performed by super imposing the modeled structure and the template structures using the molecular visualization software PYMOL and Chimera to calculate Root Mean Square deviation.[17]

### *Design of Inhibitors*

The structure of phytochemical compounds is built using CHEMSKETCH and saved in .mol files for further proceedings. Two types of inhibitors belonging to different category via, Commercial drugs and phytochemical compounds were chosen for the present study which is taken from Drug Bank and Pubchem database.

### *Molecular Docking*

The target protein was imported on workspace. The grids were generated and the active site of the protein was identified. The active site of the target protein was LEU 38 and GLN 39 which is the binding site of the target protein was the grid is formed for docking of the ligand in Auto Dock program. The hydrogen bond,

docking score, docking energy was calculated by this program. By using another program named Docking Server which offers a web-based, easy to use interface that handles all aspects of molecular docking from ligand and protein set-up.[18] The use of DockingServer allows the user to carry out highly efficient and robust docking calculations by integrating a number of popular software used in *in silico* chemistry into one comprehensive web service.

## RESULTS

***Target Sequence Retrieval***

The non structural C-Chemokine receptor type 6 (CCR6) protein from Interleukin 17, which is expressed in Plaque Psoriasis, contains 374 amino acid residues was obtained from UNIPROT accession number P51684.

***Protein Sequence analysis***

The sequence of the protein C-Chemokine receptor type 6 (CCR6) was analyzed to know their characteristics such as the molecular weight, physiochemical properties and their secondary structures that are predicted using Expasy tools. (Table 1)

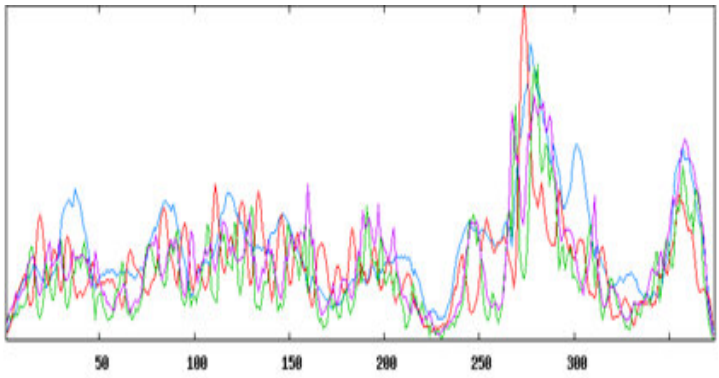


**Figure 1**
***Graphical Representation of SOPMA In Figure 1, graphical representation of SOPMA which results the alpha helix, extended strand and random coil.***

**Table 1**
***Analysis Based on Expasy tools***

| Expasy Tools | Results |
|---|---|
| Copute pI/Mw | 9.66/35670.49 |
| Protparam Aliphatic Index/GRAVY | 103.96/0.433 |
| SOPMA (alpha helix, extended strand, random coil) | 45.45%, 21.39%, 25.94% |

***Template Identification***

Standard protein-protein BLAST was used to identify the template for the protein sequence was obtained in Protein Data Bank. BLAST search resulted four best templates for CCR6 protein was selected on the basis of their identity. The template structure 2LNL which shares identity of 44%, 4MBS which shares identity of 30%, 4YAY and 4ZUD shares 36% of identity was selected as templates for homolog the non-structure protein of CCR6. In Figure 2, shows BLAST search showing the template selection based on their identity score.

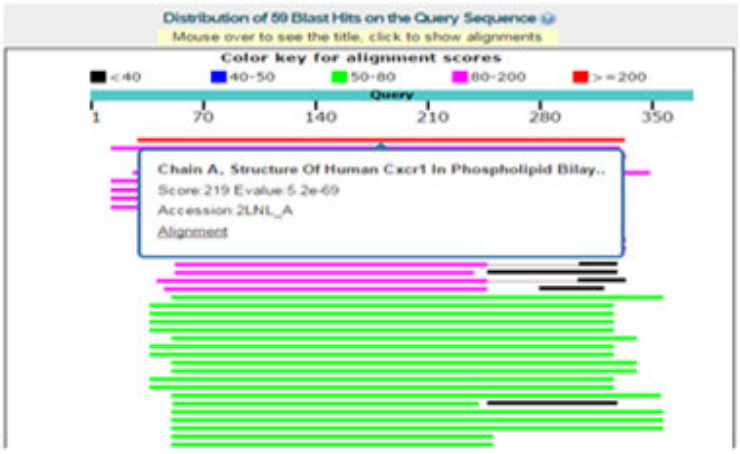


**Figure 2**
***BLAST search query for template***

***Homology Modeling of CCR6***

Modeller 9.17 was used for multiple template modeling. The software first aligns the template sequences and gives the output file with .ali extension. This was done using the script salign.py. The .ali file was further aligned with the target sequence and the side chains and backbone of the target was built based on these four template structures. This was done using the script model_mult.py. Five best models were generated and evaluated based on their DOPE (Discrete Optimized Potential Energy) score. (Table 2). The model has been submitted in Protein Model Database (PM0080736).

**Table 2**

***DOPE scores for the Homolog Model by Modeller 9.17***

| Models | DOPE Score |
|---|---|
| 1 | -35202.44531 |
| 2 | -34444.56641 |
| 3 | -34695.85547 |
| 4 | **-35403.04688** |
| 5 | -34417.73828 |

**Since the fourth model which has the highest DOPE score of -35403.04688 when compared to other models, the fourth model was selected as best based on the DOPE score.**

***Model Verification and refinement***

The model structures was verified by PROCHECK available in Structure analysis and Verification Server (SAVS). The residues PHE101 of CCR6 protein is in the disallowed region lies on the loop segment that connects the N terminal with the C terminal domain.The Refinement was done with modloop server (http://modbase.compbio.ucsf.edu/modloop/server).After loop refinement Ramachandran Plot shows all of the residues in the protein model are allowed region is 16, favored regions is 346 and outlier region is 10. Figure 3 shows the Ramachandran plot of the protein CCR6. After verification the refined target protein model has been submitted in Protein Model Database (PM0080736). Superimposition of the modeled target protein with the template 2LNL and 4MBS, which shows the identity of 44%, was predicted shown in Figure 4.

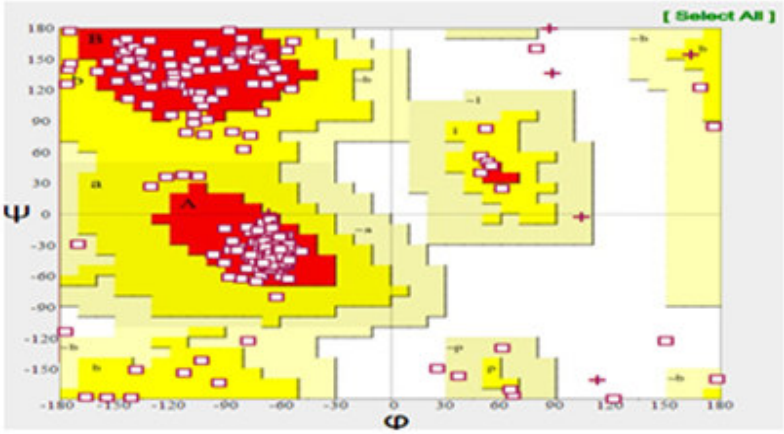


**Figure 3**

***Ramachandran Plot for the protein CCR6***

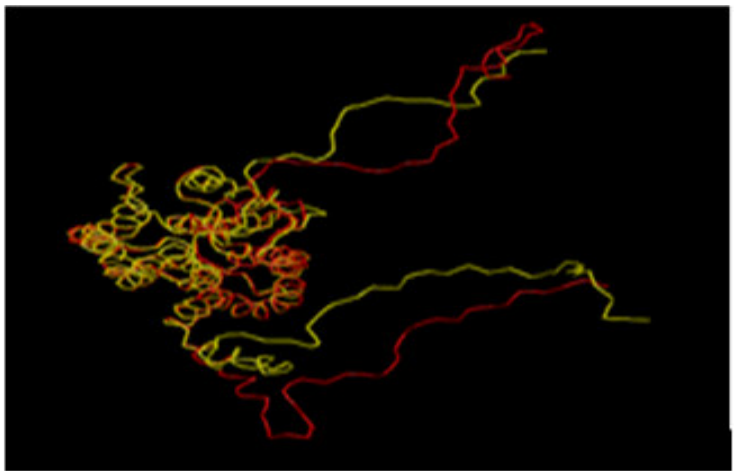

**Figure 4**

***Super imposition of Modeled target protein CCR6 and template (red – modeled target protein, yellow – templates)***

***Structure – Structure Alignment***

CCR6 target protein active site was predicted using 3D LigandSite tool which aligns and found the active site of the target having 2 binding pockets. Structure alignment of the modeled target protein and the template sequence was aligned using CHIMERA and ClustalW which shows the active site residues of the target protein CCR6 in figure 5 and figure

**Figure 5**
***Alignment of the target protein and template sequence.***

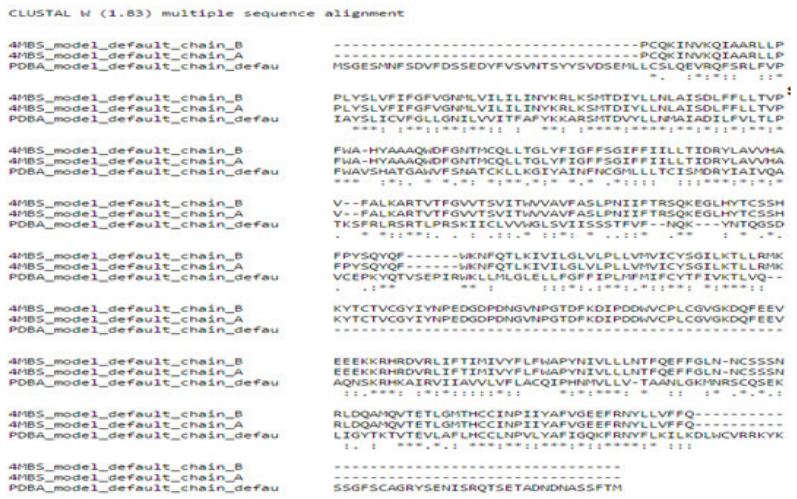

**Figure 6**
***Multiple sequence alignment shows the conservation of active sites LEU 38 and GLN39 through CLUSTALW***

***Identification of Active Site Residues***

Active site of the target protein was identified using 3D LigandSite, which predicts the active site as LEU38 and GLN 39 shown in Figure 7.

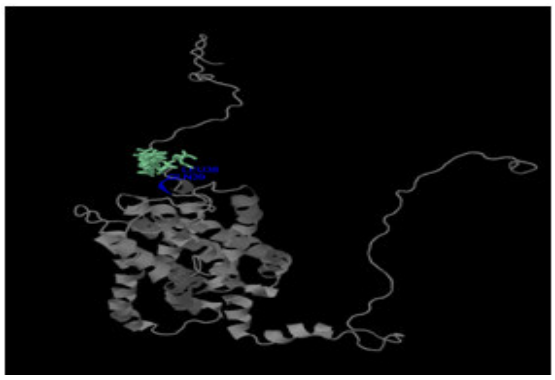

**Figure 7**
***Active site of the target protein***
***(Blue color label – LEU 38 and GLN 39)***

***Molecular Docking***

The induced fit docking was done using Auto Dock and Docking Server which shown in the Table 3 the target protein CCR6, commercial drugs and phytochemical compounds, are studied for the hydrogen bond, electrostatic energy and energy. Docking of the target protein and ligands curcumin and Calcipotriene are shown in figure 8 and 9

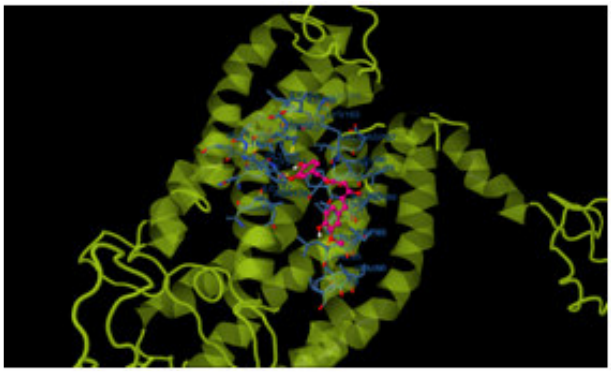

**Figure 8**
***Docking of Curcumin with target protein***
***(green color- modeled protein, pink color- Curcumin ligand molecule )***

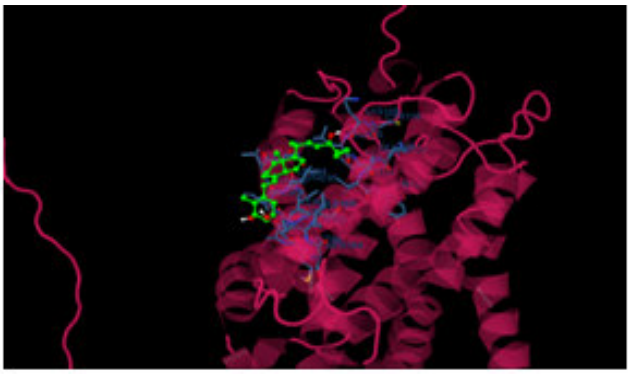

**Figure 9**
***Docking with Calcipotriene***
***(pink- modeled target protein, green – Calcipotriene ligand molecule)***

**Table 3**
***Docking results of the Commercial and phytochemical compounds with target protein***

| Compounds | H-Bond | Electrostatic energy | Energy kcal/mol |
|---|---|---|---|
| Andographolide | 3 | 0.03 kcal/mol | -7.00 |
| Curcumin | 2 | +0.05 kcal/mol | -8.49 |
| Betulin | 2 | -0.00 kcal/mol | -7.64 |
| Betulinic acid | 2 | +0.00 kcal/mol | -7.84 |
| Calcipotriene | 2 | -0.15 kcal/mol | -8.51 |
| Tazarotene | 2 | -0.03 kcal/mol | -8.35 |
| Anthraderm | 1 | -0.01 kcal/mol | -7.26 |
| Acitretin | 1 | +0.01 kcal/mol | -7.95 |

## DISCUSSIONS

Due to the lack of three-dimensional structure of the target protein CCR6 that encodes, plaque psoriasis, homology modeling has been performed to build the target protein for structure based drug design. The Ramachandran plot for the target CCR6 shows, 98% residues in allowed region suggesting that the quality of the model is good. Structure-structure alignment reveals low RMS deviation 0.3Å for the target protein. The active site of CCR6 protein is located in grid of two at the residues LEU 38 and GLN 39 are the binding pockets for the modeled protein target. Superimposition of the modeled target protein shows similar as template protein. Based on the treatment used for plaque psoriasis, drug compounds were selected which commonly used. Rather commercial drugs, naturally available drugs from the plant source were selected and molecular docking studies have been done. Calcipotriene interacted with CCR6 and gives minimum binding energy -8.51 kcal/mol and inhibition constant 2.58 µm, it also shown H–bond interaction with the target (Table-3), Therefore the above mentioned inhibitors will block the production of cytokines and inhibit the overproduction of skin cells which eases the inflamed, scaly areas.

kcal/mol and inhibition constant 35.27uM, it also shown that in has 2 H-bond interaction with the target CCR6.

Curcumin, by lowering phosphorylase kinase levels in psoriatic epidermis, has been shown to result in resolution of psoriasis, and achieves this through decreasing the population of cells capable of dividing, within the epidermis.[19] Extensive research over the last half century has revealed several important functions of curcumin. It binds to a variety of proteins and inhibits the activity of various kinases. By modulating the activation of various transcription factors, curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins.[20] Thus, Curcumin is an active herbal ingredient possessing surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activity. Optimum curcumin dosage per day is around 1200 mg per day.[21]

## CONCLUSION

Curcumin has shown therapeutic potential against a number of human diseases. Common to all of these studies have been the safety, tolerability, and non-toxicity of this polyphenol Curcumin; the active ingredient in turmeric also has the ability to alter gene expression and turmeric's ability to alter TNF cytokine expression. This is the likely reason some patients find it helpful in minimizing psoriasis. The underlying mechanism for curcumin's clinical efficacy seems to be modulation of numerous signaling molecules. Taken together, curcumin, with high efficacy and safety, has a great potential to treat psoriasis.

## CONFLICT OF INTEREST

Conflict of interest declared none.

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