EVALUATION OF ANTI-INFLAMMATORY, ANTI-ALLERGIC AND IMMUNE MODULATORY POTENTIAL OF SIDDHA FORMULATION OMA LEGIUM BY COMPUTATIONAL DOCKING ANALYSIS

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ABSTRACT
Scabies commonly called as sirangu in tamil is a contagious skin infestation that affects an estimated 300 million people around the world every year. Current medications commonly prescribed for scabies like permethrin offers increased adverse effect. Hence it a need of the hour to explore the alternate treatment strategy from Indian traditional medicine like siddha which has several formulations comprises of herbal lead in clinical management of this chronic inflammatory condition. It is evident that the prostaglandin and histamine plays a major role in arising the symptoms of scabies such as pain, inflammation, swelling, itching etc. The main aim of the present investigation is to screen the anti-allergic and anti-inflammatory potential of phytocomponents such as Beta Pinene, Thymol and Carvacrol present in the formulation Oma legium (OL) against target histamine, cyclooxygenase and prostaglandin synthases receptors along with their respective standards using computational docking analysis. The results of the study indicates that the lead Beta Pinene, Thymol and Carvacrol possess significant inhibitory action against Histamine, Prostaglandin Synthase and cyclooxygenase II, whereas the compounds Thymol has no activity against cyclooxygenase I. Based on the results of the In-silico screening analysis it was concluded that the compound’s such as Beta Pinene, Thymol and Carvacrol present in the siddha formulation OL possess significant inhibition of inflammatory mediators therefore this formulation may have promising immuno modulatory activity and may effective against inflammatory condition like scabies.

KEYWORDS: Scabies, Siddha, Oma legium, Histamine, Cyclooxygenase, Prostaglandin synthases, Immuno modulatory activity.

1. INTRODUCTION
Scabies is affecting the people at all socioeconomic levels, individuals who are young, elderly, immunocompromised or developmentally delayed are at significantly higher risk for scabies and related complications.[1-2] Community-wide and institutional outbreaks[3] have occurred in child care settings, long-term care facilities and prisons. Because scabies is associated with poverty, overcrowding, malnutrition and reduced access to health care[4], some Indigenous and resource-poor communities are disproportionately impacted.[5]

Scabies is more common in women than in men, which agreed with our results and more common in winter than summer. Scabies is very easy to misdiagnose because early subtle cases may look like small pimples or mosquito bites. Over a few weeks, however, mistakes like this become evident as patients feel worse and worse with symptoms they can't ignore.[6]

Medications commonly prescribed for scabies include permethrin cream. Permethrin is a topical cream that contains chemicals that kill scabies mites and their eggs. The most common side effects on sustainable usage of permethrin include tingling, numbness and skin rashes. Hence it is right time to explore the alternate remedy for management of scabies associated complications like inflammation, swelling, itching and pain.

Siddha is one of the oldest systems of medicine practiced in the South India. Novel siddha formulations like Oma
Legium consist of unique blend of poly herbs which is effective against clinical management of scabies associated symptoms such as pain, itching, swelling and allergy.

The primary healthcare benefits of using such plant-derived siddha formulations are relatively safer when compared to allopathic drugs and offer profound therapeutic benefits.\(^7\)  Single and polyherbal preparations have diverse range of bioactive molecules and play a dominant role in the maintenance of human health since ancient times.\(^8\) More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines.\(^9\)

Due to the rising ethical issues on the usage of laboratory animals against screening of drugs for scabies made researcher to acquire alternate high precision techniques like virtual screening. Molecular Docking continues to hold great promise in the field of Computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein. So result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 - 80% of ligands correctly.\(^10\)

Advanced docking methods may be used to improve results in cases where the limitations of requiring a rapid method for energy evaluation are too restrictive. For instance, many docking methods employ a rigid model for the receptor, which often leads to improper results for proteins with appreciable induced fit upon binding. Auto Dock includes a method for treating a selection of receptor sidechains explicitly, to account for limited conformational changes in the receptor.\(^11\) In addition, ordered water molecules often mediate interactions between ligands and receptors and advanced methods for treating selected waters explicitly have been implemented in Auto Dock. Both of these advanced methods are demonstrated in this protocol. The main of the present investigation is to investigate the anti-inflammatory and anti-allergic potential of the phytoconstituents present in the formulation Oma Legium by computational docking analysis.

### 2. MATERIALS AND METHODS

#### 2.1. Source of raw drugs

The herbs is collected from southern zone of Tamil Nadu and other required raw drug is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India. All the herbs were authenticated by the Pharmacognosist, SCRI Chennai, Tamil Nadu, India.

#### 2.2. Ingredients

The siddha formulation Oma Legium comprises of the following herbs as phyto ingredients

1. Omam (Tachyspermum ammi) - 3500gms
2. Amuukkurak-kizhangu (Withania Somnifera) - 35gms
3. Stink (Shorea Robusta) - 35gms
4. Parangi-pattai(Smilax china) - 35gms
5. Karpokaarisi –(PsoraleaCorylifolia) - 35gms
6. Sugar - 350grm
7. Nei - 2lit

#### 2.3. Software’s required

Several docking tools were been used in recent times which works behind structure-based drug design strategies one among which is auto dock 4 a componentional software tools used to analyze the protein 3RZE, 1IGX, 3KK6, 6COX and to study the binding energy properties with the following lead component such as Beta Pinene, Thymol, Carvacrol along with standard Cetirizine, Ibuprofen, Celecoxib and Salicylic acid. Histamine 1 receptor with PDB code 3RZE, Prostaglandin H2 synthase with PDB 1HGX, Cyclooxygenase II with PDB 6COX and Cyclooxygenase I with PDB 3KK6 was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with standard at the active site 3D space of enzyme of interest DPP-4 using online DOCKING SERVER web tool module.

#### 2.4. Ligand preparation

The ligands such as Beta Pinene, Thymol, Carvacrol along with standard Cetirizine, Ibuprofen, Celecoxib and Salicylic acid built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.
Table 1: Ligand Properties of the selected Lead.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molar weight g/mol</th>
<th>Molecular Formula</th>
<th>H Bond Donor</th>
<th>H Bond Acceptor</th>
<th>Rotatable bonds</th>
<th>Log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Pinene</td>
<td>136.238</td>
<td>C10H16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.1</td>
</tr>
<tr>
<td>Thymol</td>
<td>150.221</td>
<td>C10H14O</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>150.221</td>
<td>C10H14O</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>388.89</td>
<td>C21H25CIN2O3</td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>1.7</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>206.285</td>
<td>C13H18O2</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>381.37</td>
<td>C17H14F3N3O2S</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>3.4</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>138.122</td>
<td>C7H6O3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2.3</td>
</tr>
</tbody>
</table>


2.5. Active Site Prediction
Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein. The potential ligand binding sites in 3RZE, 1IGX, 3KK6, 6COX target protein is identified using grid space of 1 and probe of radius 5.0 angstrom. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations.
2.6. Docking Methodology
Docking calculations were carried out using Docking Server\textsuperscript{[15,16]} Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Beta Pinene, Thymol, Carvacrol along with standard Cetirizine, Ibuprofen, Celecoxib and Salicylic acid and their binding affinity towards the target protein with PDB 3RZE, 1IGX, 3KK6, 6COX, as shown in figure 3 to 6.

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method\textsuperscript{[17]} Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied\textsuperscript{[18]}

3. RESULTS
3.1. Results of Binding interaction with Histamine 1 Receptor
The result of binding interactions of the ligand with Histamine 1 receptor has revealed that out of three compound’s Carvacrol has 8 interactions (53%) similar to that of the standard Citrazine hence it has Histamine 1 Receptor inhibition activity similarly other two compounds thymol has 46% percentage and Betapinene has 26% similar interaction to that of the standard hence all three compounds has promising Histamine 1 receptor inhibition activity.

Table 2: Summary of the molecular docking studies of compounds against Histamine 1 receptor with PDB code 3RZE.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*µM)</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Pinene</td>
<td>-6.24</td>
<td>26.70</td>
<td>430.26</td>
</tr>
<tr>
<td>Thymol</td>
<td>-7.10</td>
<td>6.21</td>
<td>536.04</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>-6.58</td>
<td>14.99</td>
<td>598.02</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>-7.7</td>
<td>272.11*</td>
<td>931.84</td>
</tr>
</tbody>
</table>

Fig 3: Target protein Histamine 1 receptor with PDB code 3RZE.

Fig 4: Target protein Prostaglandin H2 synthases 1IGX.

Fig 5: Target protein Cyclooxygenase I with PDB code 3KK6.

Fig 6: Target protein Cyclooxygenase II with PDB 6COX.
3.2. Results of Binding interaction with Prostaglandin Synthase
The result of binding interactions of the ligand with Prostaglandin synthase has revealed that out of three compound’s Carvacrol has 4 interactions (90%) similar to that of the standard Citrazone Salicylic acid hence it has Prostaglandin Synthase inhibition activity similarly other two compounds thymol has 40% percentage and Betapinene has 20% similar interaction to that of the standard hence all three compounds has promising Prostaglandin Synthase inhibition activity.

Table 3: Summary of the molecular docking studies of compounds against Prostaglandin H2 synthases- 1IGX.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*mM)</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Pinene</td>
<td>-3.87</td>
<td>1.45*</td>
<td>345.39</td>
</tr>
<tr>
<td>Thymol</td>
<td>-4.10</td>
<td>980.29</td>
<td>372.85</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>-3.84</td>
<td>1.52*</td>
<td>417.17</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>-1.98</td>
<td>35.44*</td>
<td>461.90</td>
</tr>
</tbody>
</table>

3.3. Results of Binding interaction with Cyclooxygenase 1 Receptor
The result of binding interactions of the ligand with Cyclooxygenase 1 has revealed that out of three compound’s Betapinene has 6 interactions (60%) similar to that of the standard Ibuprofen hence it has promising COX 1 inhibition activity similarly other compound Carvacrol has 40% percentage similar interaction to that of the standard hence both compounds has COX 1 inhibition activity. Compound Thymol has no COX1 inhibition activity.

Table 4: Summary of the molecular docking studies of compounds against Cyclooxygenase 1 with PDB 3KK6.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*mM)</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Pinene</td>
<td>-5.70</td>
<td>66.06</td>
<td>425.17</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>-6.01</td>
<td>39.44</td>
<td>594.73</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>-6.33</td>
<td>23.05</td>
<td>524.81</td>
</tr>
</tbody>
</table>
3.4. Results of Binding interaction with Cyclooxygenase 2 Receptor
The result of binding interactions of the ligand with Cyclooxygenase II has revealed that out of three compound’s Betapinene and Carvacrol has 3 interactions (60%) similar to that of the standard Celecoxib hence it has promising COX 2 inhibition activity similarly other compound Thymol has 20% percentage similar interaction to that of the standard hence all three compounds has promising COX 2 inhibition activity.

Table 5: Summary of the molecular docking studies of compounds against Cyclooxygenase II with PDB 6COX.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*mM)</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Pinene</td>
<td>-3.99</td>
<td>1.19*</td>
<td>357.52</td>
</tr>
<tr>
<td>Thymol</td>
<td>-4.27</td>
<td>743.86</td>
<td>403.11</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>-3.17</td>
<td>4.72*</td>
<td>404.21</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>-3.70</td>
<td>1.95*</td>
<td>427.00</td>
</tr>
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</table>

4. DISCUSSION
It is evident that scabies is a highly pruritic disorder induced by an immune allergic response to infestation of the skin by the mite sarcoptes scabiei that burrows in the stratum corneum of the skin. Scabies is a neglected worldwide health problem. In the U.S., it occurs frequently in the general population and in institutions such as daycare centers and nursing homes. The prevalence is nearly 100% in infants and >50% in older children and women in some populations in the world. Scabies is more prevalent in children and young adults.[19]

There are several complications associated with scabies which includes inflammatory response of the host immune system on to the dermal layer of the affected individuals. Inflammatory mediators like prostaglandins, histamines plays a key role in triggering the episodes of pain, swellings, itching etc. The enzymes cyclooxygenase I & II, prostaglandin synthase controls the synthesis of such inflammatory mediators.[20]

Evaluation of anti-scabies activity in animals is tedious process and henceforth to justify the potential of the drug in-vivo it is now days essential to prove the efficacy by In-silico or by Invitro as per regulatory guidelines and hence this present study was attempted to provide alternate screening therapy for evaluating the anti-histamine, anti-inflammatory and analgesic activity of the selected formulation oma legium. According to the literature the herbs used for preparing this formulation comprises of phytocomponents such as Beta Pinene, Thymol and Carvacrol. 3D structure of this phytocomponents were docked with protein target like Histamine 1 receptor with PDB code 3RZE, Prostaglandin H2 synthase with PDB 1IGX, Cyclooxygenase II with PDB 6COX and Cyclooxygenase I with PDB 3KK6.

Computational docking can be used to predict bound conformations and free energies of binding for small molecule ligands to macromolecular targets. Docking is widely used for the study of biomolecular interactions.
and mechanisms, and is applied to structure-based drug design. The methods are fast enough to allow virtual screening of ligand libraries containing tens of thousands of compounds. This protocol covers the docking and virtual screening methods provided by the AutoDock suite of programs, including a basic docking of a drug molecule with an anticancer target, a virtual screen of this target with a small ligand library, docking with selective receptor flexibility, active site prediction and docking with explicit hydration.\textsuperscript{[21]}

Prostaglandin plays an ultimate role in the pathology of skin inflammatory condition like scabies. Enzyme cyclooxygenase (COX) plays an important role in many cellular processes. It is necessary for a synthesis prosta glandins, which belong to the most important mediators of inflammatory and pain processes.\textsuperscript{[22]}

The result of binding interactions of the ligand with Cyclooxygenase I has revealed that out of three compound’s Betapinene has 6 interactions (60%) similar to that of the standard Ibuprofen hence it has promising COX 1 inhibition activity similarly other compound Carvacrol has 40% percentage similar interaction to that of the standard hence both compounds has COX 1 inhibition activity. Compound Thymol has no COX1 inhibition activity.

Many studies show an importance of COX not only in periphery but in central nervous system as well. Inhibitors of the COX I and II, the nonsteroidal anti-inflammatory drugs (NSAIDs), relieve inflammatory pain, but are associated with gastrointestinal and cardiovascular complications. Given the widespread use of NSAIDs, there has been a longstanding interest in optimizing their risk-benefit ratio.\textsuperscript{[23]}

The result of binding interactions of the ligand with Cyclooxygenase II has revealed that out of three compound’s Betapinene has 6 interactions (60%) similar to that of the standard Celecoxib hence it has promising COX 2 inhibition activity similarly other compound Thymol has 20% percentage similar interaction to that of the standard hence all three compounds has promising COX 2 inhibition activity.

Prostaglandin synthase is an biologically significant enzyme catalyzing the initial steps in the synthesis of prostaglandins from arachidonic acid; it comprises the enzyme catalyzing the initial steps in the synthesis of Prostaglandin synthase is an biologically significant compound has promising COX 2 inhibition activity similarly other two compounds have 60% similar interaction to that of the standard hence all three compounds has promising Histamine 1 receptor inhibition activity.

The important roles of histamine in body physiology and various pathologic events have been well established. Histamine is not only the major mediator of the acute inflammatory and immediate hypersensitivity responses, but has also been demonstrated to affect chronic inflammation and regulate several essential events in the immune response. The diverse effects of histamine on immune regulation are due to the differential expression and regulation of four histamine receptors (HR) and their distinct intracellular signals.\textsuperscript{[24]}

The result of binding interactions of the ligand with Histamine 1 receptor has revealed that out of three compound’s Carvacrol has 6 interactions (53%) similar to that of the standard Citrazine hence it has Histamine 1 Receptor inhibition activity similarly other two compounds thymol has 46% percentage and Betapinene has 26% similar interaction to that of the standard hence all three compounds has promising Histamine 1 receptor inhibition activity.

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5. CONCLUSION

Based on the results of the computational analysis it was concluded that the compound’s such as Betapinene, Thymol, Carvacrol present in the formulation Oma Legium possess significant inhibition of COX 1& 2, Prostaglandin synthases and Histamine 1blocking activity further it was concluded that this formulation may have promising anti-inflammatory, anti-allergic, immune modulatory activity.

6. REFERENCES