ANTI VITILIGO ACTIVITY OF SIDDHA FORMULATION OMA LEGIUM – AN IN VITRO STUDY

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ABSTRACT

Background: Vitiligo is a common acquired disorder of skin pigmentation characterized by localized loss of skin pigments secondary to melanocytes damage. It affects male and female equally. Aim: To investigate the anti vitiligo activity of the siddha formulation of oma legium.

Materials and Methods: Crystalline structure of the target protein Tyrosinase with PDB 1WX3 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

Results: Oma legium had Withaferin A, Asiatic acid, Kaempferitrin, Isovitexin, Carvone and Astragalin present in the Siddha formulation Oma legium reveals significant binding against the target protein by interacting with amino acid present on the active site of the tyrosinase enzyme.

Conclusion: The present study revealed that the Siddha formulation oma legium had anti vitiligo activity established through In-vitro study.

Keyword: Siddha, Oma legium, Anti vitiligo activity, In-vitro study.

1. Introduction

Siddha, the traditional system of medicine is widely being practiced in the Tamil Nadu and the concept pertaining to drug ingredients are from plant, mineral, metals and animal origin. Legium is one of the 32 types of internal medicine. Oma legium is one among the legium used in the treatment of venpulli (vitiligo) in children. It contains Omam, Amukkura kizhangu, Kukil, Parangipattai. I have selected formulation oma legium from the text book of Athmarakshamirtham ennum vaidhiya sarasangiragam. An important objective of traditional medicine is prevention is better than cure which means prevention from disease is better than treating the disease. In the siddha system of medicine, many herbs and medicinal formulations have been reported in treating skin diseases. The ingredients of this formulation possess Antioxidant, Immunomodulatory, dedoxification of aflatoxin activity, Anti-inflammatory and Antidepressant effects.
2. Materials and Methods

Ingredients of oma legium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachyspermum ammi</td>
<td>3500g</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>35g</td>
</tr>
<tr>
<td>Shorea robusta</td>
<td>35g</td>
</tr>
<tr>
<td>Smilax china</td>
<td>35g</td>
</tr>
<tr>
<td>Psoralea corylifolia</td>
<td>35g</td>
</tr>
<tr>
<td>Saccharum officinarum</td>
<td>350g</td>
</tr>
<tr>
<td>Ghee</td>
<td>1.34 Litre</td>
</tr>
</tbody>
</table>

3. Collection and Authentication of raw drugs

All the drugs were purchased from Ramasamy chettiya raw drug store, Paris’s corner, Chennai, and the raw drugs were authenticated by the medicinal botanist of National Institute of Siddha and the mineral drug was authenticated by Gunapadam laboratory in charge. The medicine was prepared as per Sasthric Siddha Literature in Gunapadam laboratory of National Institute of siddha after proper purification. The prepared medicine was authenticated by the guide and the lab in charge for its completeness.

4. Method and purification

Raw drugs were purified as per the purification method described in text book of Sarakku Suthi Muraigal. All the drugs were purified in Gunapadam laboratory of National Institute of Siddha.

- **Omam**
  - It is purified by soaking it in lime stone water and then it is dried.

- **Amukkura kizhangu**
  - It is dried and powdered. Milk is taken in a vessel and the mouth of the vessel is covered with a cloth. The powdered Amukkara kilangu is placed over the cloth and then it is boiled for 3 hours and then dried.

- **Kukil**
  - It is soaked in thripala decoction for 6 hours.

- **Parangipattai**
  - It is purified by cleaning it with pure cloth and the outer layer is removed.

- **Karpogari**
  - It is soaked in the juice of Ocimum basilicum and then dried.

- **Sarkkarai**
  - It is crushed and grinded finely.
5. Preparation

Omam is purified and mixed with 21.5 litres of water and reduced to 1/8 in decoction form. Sugar is added to the decoction to get the Pagu Padham consistency. The other raw drugs purified and powdered are added to the ghee and is mixed well until the texture is obtained.

6. ANTI-VITILIGO STUDY

List of herbs present in the formulation

- *Trachyspermum ammi*
- *Withania somnifera*
- *Shorea robusta*
- *Smilax china*
- *Psoralea corylifolia*

List of Phytocomponents Selected for docking

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Phyto components</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trachyspermum ammi</em></td>
<td>Thymol</td>
</tr>
<tr>
<td></td>
<td>Carvone</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>Withaferin A</td>
</tr>
<tr>
<td><em>Shorea robusta</em></td>
<td>Asiatic acid</td>
</tr>
<tr>
<td><em>Smilax china</em></td>
<td>Kaempferitin</td>
</tr>
<tr>
<td><em>Psoralea corylifolia</em></td>
<td>Isovitexin</td>
</tr>
<tr>
<td></td>
<td>Bavachinin</td>
</tr>
<tr>
<td></td>
<td>Astragalin</td>
</tr>
</tbody>
</table>

7. Objective

The main objective of the study is to find the lead molecules to bind with these core bio active amino acid residues His38, His54, and His63, His190, His194 and His216 which mediates the enzymatic action of the enzyme called tyrosinase thereby it tend to enhance / synergies the action of tyrosinase enzyme to improve the action of melanogenesis. In general melanin pigment production which was actually found to be deprived in hypopigmentation medical condition like vitiligo, so improving tyrosinase activity helps to achieve the melanogenesis in condition like vitiligo.

<table>
<thead>
<tr>
<th>PDB</th>
<th>Name of the Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>1WX3</td>
<td>Tyrosinase</td>
</tr>
</tbody>
</table>
RECEPTOR STRUCTURE

Crystalline structure of the target protein Tyrosinase with PDB 1WX3 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

8. METHODOLOGY

Docking calculations were carried out using Auto Dock 4. 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 for the retrieved phytocomponents against the target protein. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell et al., 1998). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998). AutoDock 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 & Wets local search method (Solis and Wets, 1981). 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.
2D and 3D Structure ofSelected Ligands

Thymol

Carvone

Withaferin A

Asiatic acid

Kaempferitrin
Docking Pose

Thymol with Tyrosinase– PDB- 1WX3

2D Interaction Plot

Hydrogen bond plotting
Analysis with core amino acid
Carvone with Tyrosinase – PDB- 1WX3

2D Interaction Plot
Hydrogen bond plotting
Analysis with core amino acid

Withaferin A with Tyrosinase – PDB- 1WX3

2D Interaction Plot
Hydrogen bond plotting
Analysis with core amino acid
Asiatic acid with Tyrosinase – PDB- 1WX3

Kaempferitin with Tyrosinase – PDB- 1WX3

Isovitexin with Tyrosinase – PDB- 1WX3
2D Interaction Plot

Hydrogen bond plotting
Analysis with core amino acid

Bavachinin with Tyrosinase – PDB- 1WX3

2D Interaction Plot

Hydrogen bond plotting
Analysis with core amino acid

Astragalin with Tyrosinase – PDB- 1WX3
Ligand Properties of the Compounds selected for docking against Tyrosinase (1WX3)

<table>
<thead>
<tr>
<th>Compound</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>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol</td>
<td>150.221 g/mol</td>
<td>C10H14O</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carvone</td>
<td>150.221 g/mol</td>
<td>C10H14O</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Withaferin A</td>
<td>470.6 g/mol</td>
<td>C28H38O6</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Asiatic acid</td>
<td>488.7 g/mol</td>
<td>C20H28O4</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Kaempferitin</td>
<td>286.24 g/mol</td>
<td>C15H10O6</td>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Astragalin</td>
<td>448.4 g/mol</td>
<td>C21H30O11</td>
<td>7</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Bavachinin</td>
<td>338.4 g/mol</td>
<td>C21H22O4</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Isovitexin</td>
<td>432.4 g/mol</td>
<td>C21H26O10</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

Summary of the molecular docking studies of compounds against Tyrosinase (1WX3)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Binding Free energy Kcal/mol</th>
<th>Inhibition constant Ki µM (*mM)(**nM)</th>
<th>Electrostatic energy Kcal/mol</th>
<th>Intermolecular energy Kcal/mol</th>
<th>Total Interaction Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol</td>
<td>-4.46</td>
<td>533.56</td>
<td>-0.07</td>
<td>-5.02</td>
<td>453.11</td>
</tr>
<tr>
<td>Carvone</td>
<td>-4.80</td>
<td>302.53</td>
<td>-0.05</td>
<td>-5.10</td>
<td>458.94</td>
</tr>
<tr>
<td>Withaferin A</td>
<td>-6.43</td>
<td>19.52</td>
<td>-0.09</td>
<td>-6.68</td>
<td>723.81</td>
</tr>
<tr>
<td>Asiatic acid</td>
<td>-6.70</td>
<td>12.34</td>
<td>-0.32</td>
<td>-5.30</td>
<td>690.73</td>
</tr>
<tr>
<td>Kaempferitin</td>
<td>-7.37</td>
<td>3.96</td>
<td>-0.07</td>
<td>-7.36</td>
<td>689.83</td>
</tr>
<tr>
<td>Astragalin</td>
<td>-6.26</td>
<td>25.98</td>
<td>-0.09</td>
<td>-6.49</td>
<td>747.73</td>
</tr>
</tbody>
</table>
## 9. Observation and Inference

Total of 8 bioactive lead compounds were retrieved from the herbs present in the siddha formulation omalegium. From reported data of the herb, the leads such as Withaferin A, Asiatic acid, Kaempferitrin and Isovitexin possess 60-100% binding efficacy by interacting with core target amino acids (His38, His54, and His63, His190, His194 and His216) present on the protein – Tyrosinase enzyme followed by which the compounds such as Carvone and Astragalin possess 50% binding efficacy by interacting with target amino acids.

## 10. Conclusion

Based on the results of the computational analysis it was concluded that the bio-active compound’s like Withaferin A, Asiatic acid, Kaempferitin, Isovitexin, Carvone and Astragalin present in the siddha formulation omalegium reveals significant binding against the target protein by interacting with amino acid present on the active site of the tyrosinase enzyme thereby it was concluded that these compounds may exerts promising anti-vitiligo property by synergizing the action of tyrosinase enzyme to improve the melanogenesis so that in turn improves melanin pigment production which was actually found to be deprived in hypopigmentation medical condition like vitiligo.
11. Reference


