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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 proteinTyrosinase 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.Meterials and Methods

Ingredients of oma legium

Omam	*Trachyspermum ammi*	3500g
Amukkura kizhangu	*Withania somnifera*	35g
Kukil	*Shorea robusta*	35g
Parangipattai	*Smilax china*	35g
Karbogarisi	*Psoralea corylifolia*	35g
Sarkkarai	*Saccharum officinarum*	350g
Nei	*Ghee*	1.34 Litre

3.Collection and Authentication of raw drugs

All the drugs were purchased from Ramasamy chettiyar 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

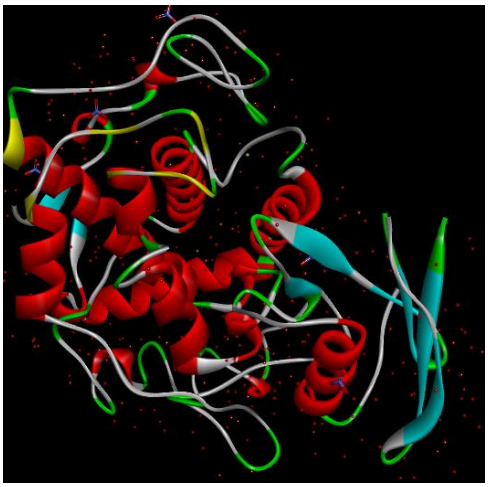
Scientific Name	Phyto components
Trachyspermum ammi	Thymol Carvone
Withania somnifera	withaferin A
Shorea robusta	Asiatic acid
Smilax china	Kaempferitrin
Psoralea corylifolia	Isovitexin Bavachinin Astragalin

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,His 190, 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.

PDB	Name of the Target
1WX3	Tyrosinase

Tyrosinase(1WX3)



RECEPTOR STRUCTURE

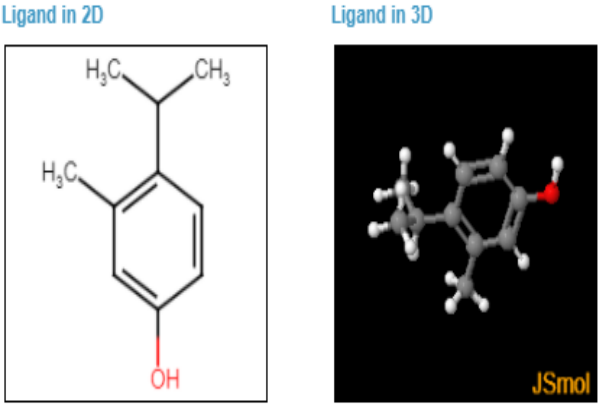
Crystalline structure of the target proteinTyrosinase with PDB 1WX3 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock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

8.METHODOLOGY

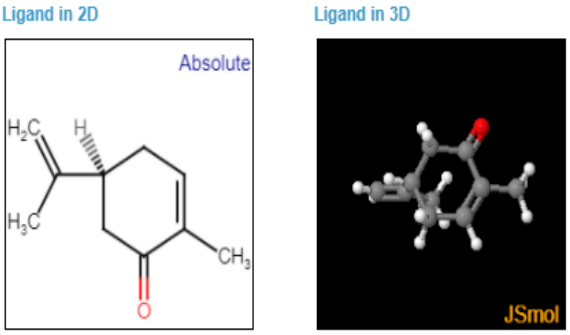
Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out for the retrieved phytocomponents against the target protein. Essential hydrogen 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 of Selected Ligands

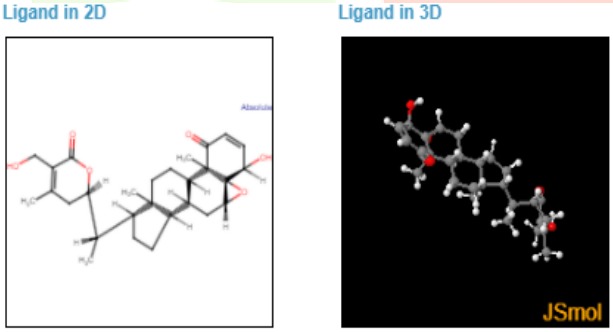
Thymol



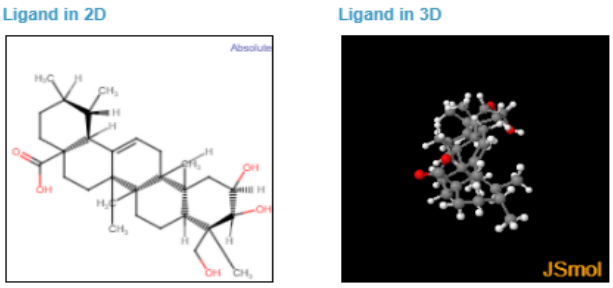
Carvone



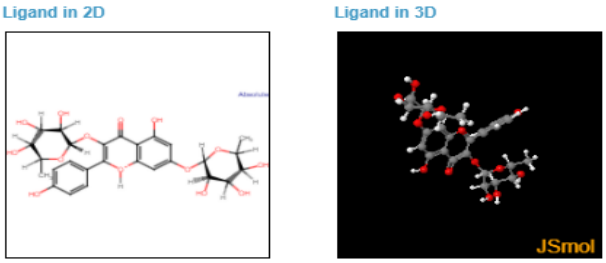
Withaferin A



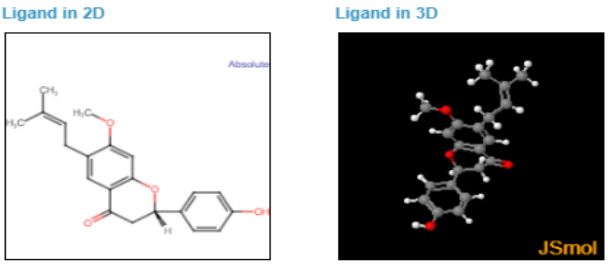
Asiatic acid



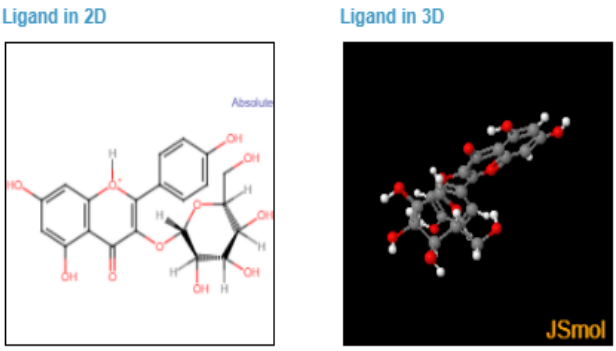
Kaempferitrin



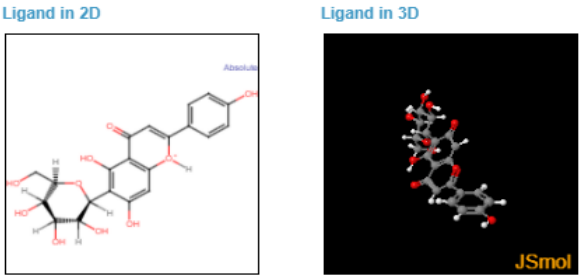
Bavachinin



Astragalin

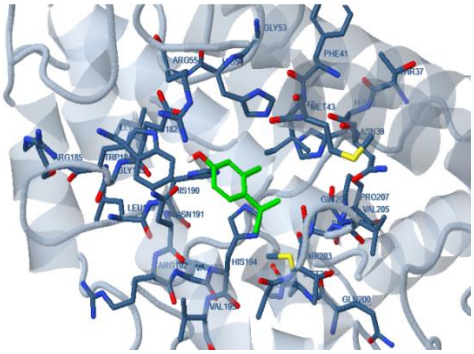


Isovitexin

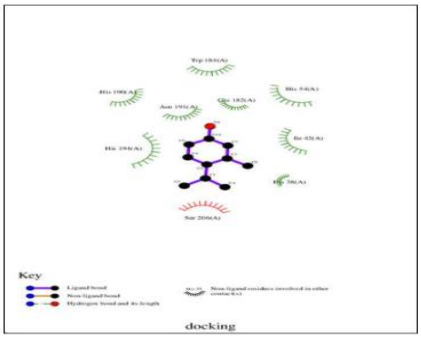


Docking Pose

Thymol with Tyrosinase– PDB- 1WX3

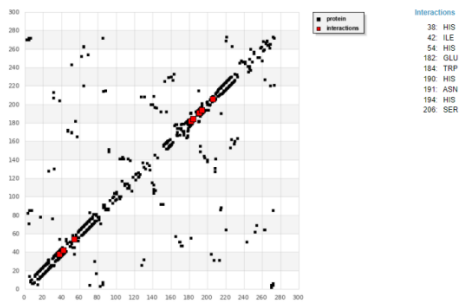


2D Interaction Plot

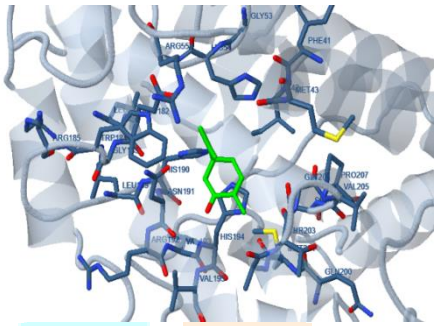


Hydrogen bond plotting

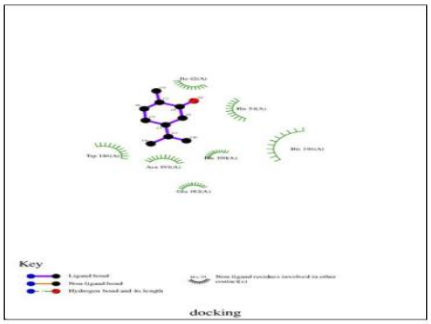
Analysis with core amino acid



Carvonewith Tyrosinase– PDB- 1WX3

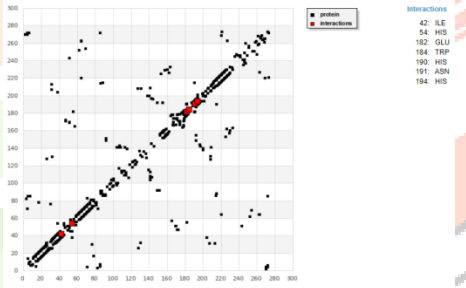


2D Interaction Plot

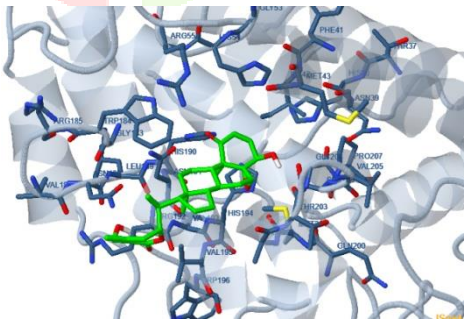


Hydrogen bond plotting

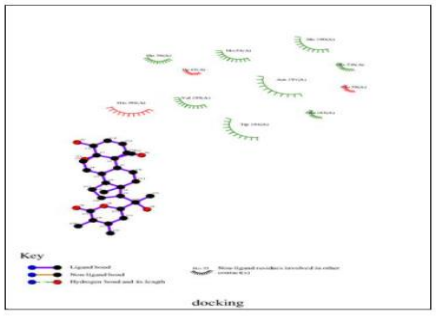
Analysis with core amino acid



withaferin Awith Tyrosinase– PDB- 1WX3

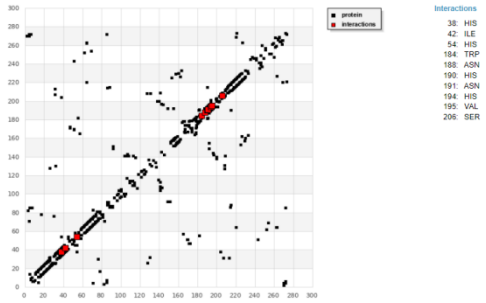


2D Interaction Plot

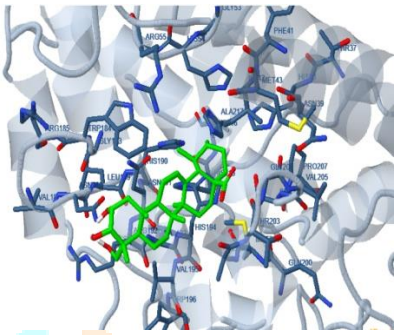


Hydrogen bond plotting

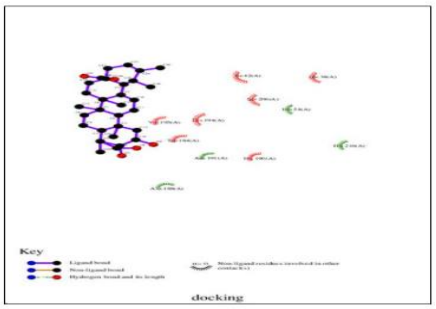
Analysis with core amino acid



Asiatic acidwith Tyrosinase– PDB- 1WX3

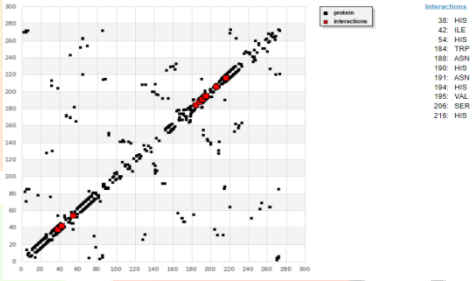


2D Interaction Plot

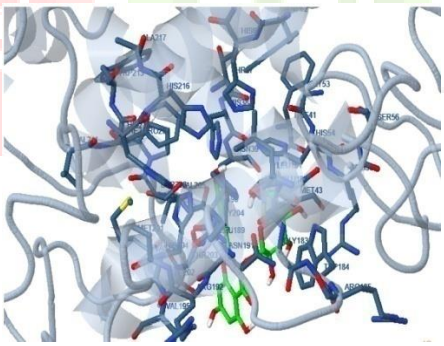


Hydrogen bond plotting

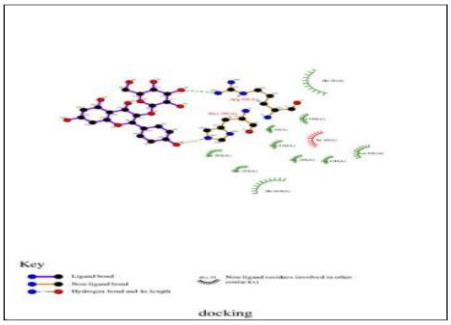
Analysis with core amino acid



Kaempferitrinwith Tyrosinase– PDB- 1WX3

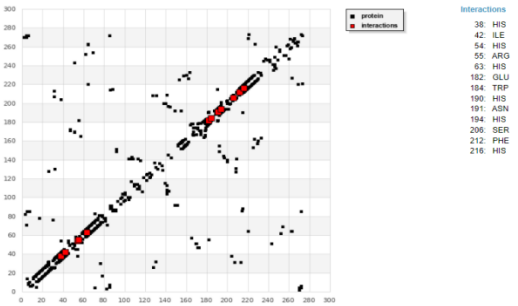


2D Interaction Plot

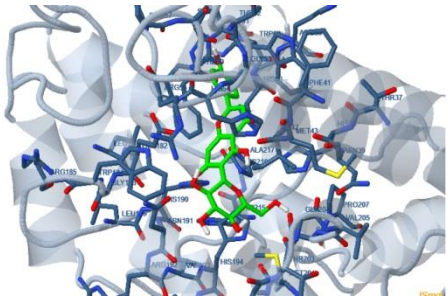


Hydrogen bond plotting

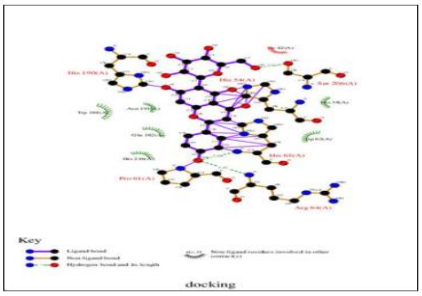
Analysis with core amino acid



Isovitexinwith Tyrosinase– PDB- 1WX3

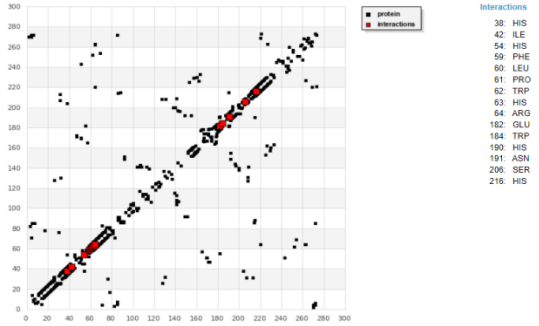


2D Interaction Plot

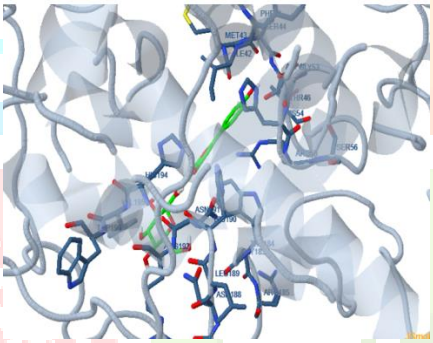


Hydrogen bond plotting

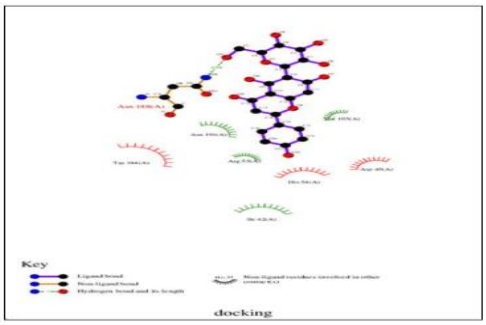
Analysis with core amino acid



Bavachininwith Tyrosinase– PDB- 1WX3

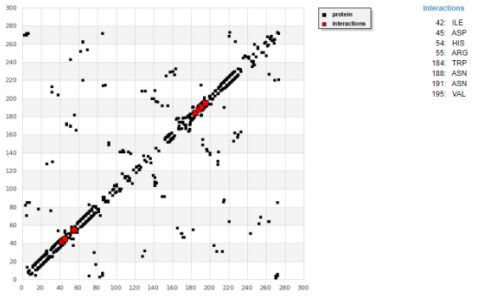


2D Interaction Plot

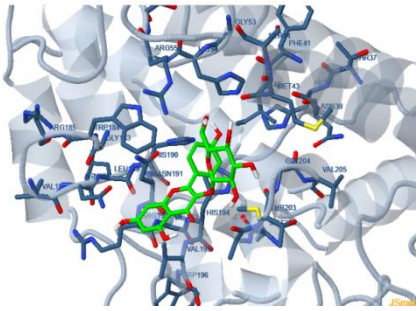


Hydrogen bond plotting

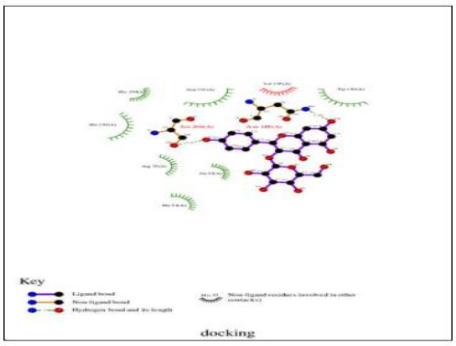
Analysis with core amino acid



Astragalinwith Tyrosinase– PDB- 1WX3

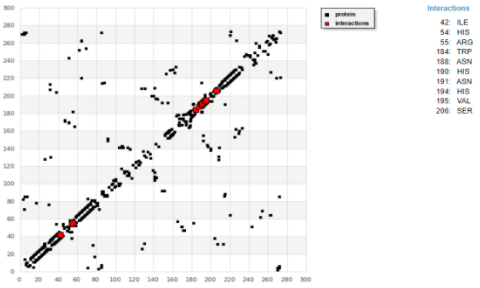


2D Interaction Plot



Hydrogen bond plotting

Analysis with core amino acid



Ligand Properties of the Compounds selected for docking against Tyrosinase (1WX3)

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Thymol	150.221 g/mol	C10H14O	1	1	1
Carvone	150.221 g/mol	$C_{10}H_{14}O$	0	1	1
Withaferin A	470.6 g/mol	$C_{28}H_{38}O_6$	2	6	3
Asiatic acid	488.7 g/mol	$C_{30}H_{48}O_5$	4	5	2
Kaempferitrin	286.24 g/mol	$C_{15}H_{10}O_6$	4	6	1
Astragalin	448.4 g/mol	$C_{21}H_{20}O_{11}$	7	11	4
Bavachinin	338.4 g/mol	$C_{21}H_{22}O_4$	1	4	4
Isovitexin	432.4 g/mol	$C_{21}H_{20}O_{10}$	7	10	3

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

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thymol	-4.46	533.56	-0.07	-5.02	453.11
Carvone	-4.80	302.53	-0.05	-5.10	458.94
Withaferin A	-6.43	19.52	-0.09	-6.68	723.81
Asiatic acid	-6.70	12.34	-0.32	-5.30	690.73
Kaempferitrin	-7.37	3.96	-0.07	-7.36	689.83
Astragalin	-6.26	25.98	-0.09	-6.49	747.73

Bavachinin	-8.23	920.45**	-0.03	-9.11	715.71
Isovitexin	-3.30	3.82*	-0.01	-2.60	595.25

Amino acid Residue Interaction of Lead and Standard against

Crystal structure of Tyrosinase – PDB 3NM8

Compound	Interactions	Amino Acid Residue- Binding												
Thymol	4	38 HIS	42 ILE	54 HIS	182 GLU	184 TRP	190 HOS	191 ASN	194 HIS	206 SER				
Carvone	3	42 ILE	54 HIS	182 GLU	184 TRP	190 HOS	191 ASN	194 HIS						
Withaferin A	4	38 HIS	42 ILE	54 HIS	184 TRP	188 ASN	190 HOS	191 ASN	194 HIS	206 SER				
Asiatic acid	5	38 HIS	42 ILE	54 HIS	184 TRP	188 ASN	190 HOS	191 ASN	194 HIS	206 SER	216 HIS			
Kaempferitrin	6	38 HIS	42 ILE	54 HIS	55 ARG	63 HIS	182 GLU	184 TRP	190 HOS	191 ASN	194 HIS	206 SER	212 PHE	216 HIS
Astragalin	3	42 ILE	54 HIS	55 ARG	184 TRP	188 ASN	190 HIS	191 ASN	194 HIS	195 VAL	206 SER			
Bavachinin	1	42 ILE	45 ASP	54 HIS	55 ARG	184 TRP	188 ASN	191 ASN	195 VAL					
Isovitexin	5	38 HIS	42 ILE	54 HIS	59 PHE	60 PRO	62 TRP	63 HIS	64 ARG	182 GLU	184 TRP	190 HOS	191 ASN	206 SER	216 HIS

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 asWithaferin A, Asiatic acid, Kaempferitrin and Isovitexinpossess 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 Astragalinpossess 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, Kaempferitrin, Isovitexin,Carvone and Astragalinpresent in the siddha formulation omalegiumrevels 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 bysynergizing the action of tyrosinase enzyme to improve the melanogenesisso that in turn improves melanin pigment production which was actually found to be deprived in hypopigmentation medical condition like vitiligo.

11.*Reference*

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