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Evaluation of Anti-vitiligo properties through an In-silico Computational screening of Selected Herbal Bioactive Components with the target protein Tyrosinase

Research Article

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Abstract

Background: Vitiligo is one of the most hypopigmented disorders of the skin, that can be correlated with *Swetha kuttam or ven kuttam or ven pulli* mentioned in Siddha classical literature. Current therapeutic management available for vitiligo is only moderately effective in controlling further severity of the symptoms. Hence in recent times, people rely most on alternative complementary treatments for the management of vitiligo. The research articles have shown the following bio-active compounds present in the selected herbs Kaempferol, Quercetin, Anacardic acid, Glabridin, Isovitexin, Aristolochic acid and Hydnocarpin which is already mentioned in the Siddha literature for the treatment of vitiligo (*venkuttam*). Objective: The study aims to perform the in-silico computational analysis of the selected herbal bioactive components against the target enzyme Tyrosinase in Vitiligo. Methods: Docking calculations were carried out for the obtained bio-active compounds such as Kaempferol from *Indigofera aspalathoides* (Vahl.ex.DC), Quercetin from *Smilax china*(Linn.), Tinosporide from *Tinospora cordifolia* (Willd.) Hook.f. & Thoms, Anacardic acid from *Semecarpus anacardium* (Linn.), Glabridin from *Glycyrrhiza glabra*(Linn.), Isovitexin from *Psoralea corylifolia* (Linn.), Oleic acid from *Nigella sativa*(Linn.), Aristolochic acid from *Aristolochia bracteolata* (Lam.) & Hydnocarpin from *Hydnocarpus laurifolia* (Dennst.) Sleumer against Tyrosinase (PDB)-1WX3. In-Silico docking simulations with a predefined algorithm were adopted to investigate the efficacy of the selected herbal components with that of the target protein of interest. Auto Dock software version 4 was utilized for docking predictions followed by visualization using drug discovery studio. Results: A total number of 9 bioactive compounds have been screened in total, the following leads as Quercetin, Glabridin, Isovitexin and Hydnocarpin which interact with target to form four interactions with 70 –100% binding efficacy by interacting with core target amino acid (His 38, His 54, His 190 & His 194) present on the protein - Tyrosinase enzyme. Conclusion: Based on the computational analysis led to the conclusion that the bioactive molecules present in the selected herbals pose significant binding efficacy and may exert promising anti-vitiligo properties.

Keywords: Hypopigmentation, Vitiligo, *Swetha kuttam,* Molecular docking, Siddha medicine, Tyrosinase.

Introduction

The term "vitiligo" refers to gradual, idiopathic hypopigmentation of the skin, mucous membranes, eyes, and hair - a kind of leucoderma that is frequently familial and is distinguished microscopically by a lack of melanocytes. The appearance of white macules or patches on the skin that are brought on by the degeneration of functional epidermal melanocytes defines this illness. It is visible to the unaided eye and can provide a wealth of information about the patient and the illness (1). Worldwide, vitiligo affects 1% to 2% of the population. In India, 0.22 to 2.5% of individuals with genetic diseases were impacted (2). It has been stated that the condition, which is characterised by a gradual, patchy loss of skin pigmentation and is caused by the selective elimination of melanocytes, is complex, with both genetic predisposition and environmental variables thought to be responsible (3). Tyrosinase is a copper-containing enzyme that the TYR gene codes for; it is an enzyme of the melanocyte that catalyses melanin production and serves as an important autoantigen in generalised vitiligo. It is located inside melanosomes

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that are made by the skin's melanocytes. It is a protein (Tyrosinase), is not a part of the immune system. The pathophysiology of vitiligo has been investigated using several significant hypotheses, among which are based on inherited, neurological, autoimmune disorders, oxidant-antioxidant mechanisms, and melanocytorrhagia. Variations that occur in more than 30 genes in different permutations has been associated with a higher probability of developing vitiligo. The genes NLRP1 and PTPN22 are two of them at all times. A protein that plays a part in the immune system and helps to control inflammation is made with the aid of the NLRP1 gene. Whenever the immune system delivers signalling compounds and white blood cells to a site affected by injury or disease to fight off microbial invaders and promote tissue regeneration, leading to inflammation. The process of inflammation is subsequently stopped (inhibited) by the body to safeguard its cells and tissues. The PTPN22 gene encodes a protein associated with signalling which assists in modulating the activity of T cells, classified as immune system cells. T cells recognise foreign molecules and defend the body against infection. It is most likely that the variations in the NLRP1 and PTPN22 genes that are linked to an increased risk of establishing vitiligo affect the functioning of the NLRP1 and PTPN22 proteins, making it more difficult for the body to manage inflammation and prevent the immune system from attacking its own tissues (4).

Signs and symptoms of *Venkuttam*, also known as *swetha kuttam*, are mentioned in the verses of *Yugi Vaithiya Chinthamani Perunool - 800*. *Venkuttam* can be present with the symptoms of the skin being white and may or may not be thickened, whitening of the hair on the body, and burning pain with non-healing ulcers in the palms, mouth, lips, and genital areas (5). *Venkuttam* can be correlated with Vitiligo in modern medical science.

Herbal remedies are known to be effective in treating hypopigmented disorders by their active compounds via synergising the action of tyrosinase enzyme to improve the melanogenesis which in turn improves melanin pigment production which was found to be deprived in hypopigmented medical conditions like vitiligo (6). The research articles have shown the major bioactive compounds present in the selected herbs such as *Indigofera aspalathoides* (Vahl.ex.DC) (7), *Smilax china* (Linn.) (8), *Tinospora cordifolia* (Willd.)Hook.f.&Thoms.(9), *Semecarpus anacardium* (Linn.) (10), *Glycyrrhiza glabra* (Linn.) (11), *Psoralea corylifolia* (Linn.) (12), *Nigella sativa* (Linn.)(13), *Aristolochia bracteolata* (Lam.) (14) & *Hydnocarpus laurifolia* (Dennst.) Sleumer (15) which are already mentioned in the Siddha literature for the treatment of vitiligo (*venkuttam*). The study aims to perform the In-Silico computational analysis of the selected herbal bio-active components against the target enzyme Tyrosinase in Vitiligo.

Objective

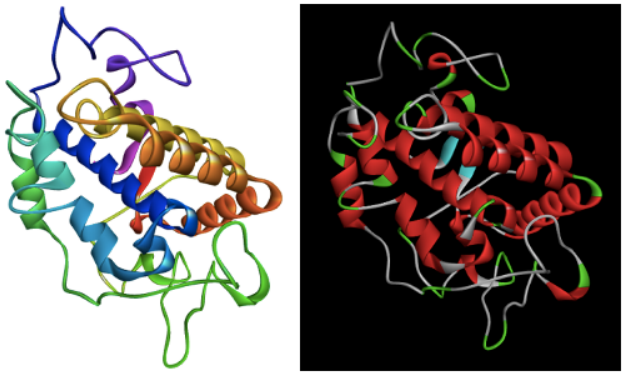
The main objective of the study is to find the lead molecules to bind with these core bioactive amino acid residues His38, His54, His63, His190, His194 and His216 which mediates the enzymatic action of the enzyme called tyrosinase, thereby tending to enhance/ synergy the action of tyrosinase enzyme to improve the action of melanogenesis. Enhancing tyrosinase activity helps to achieve melanogenesis in conditions like vitiligo because it was discovered that hypopigmentation medical conditions like vitiligo lack melanin pigment production.

Materials and Methods

Target protein Tyrosinase (PDB 1WX3) preparation

The crystalline structure of the target protein Tyrosinase with PDB-1WX3 (Fig.1) was retrieved from the protein data bank, protein clean-up process was done and essential missing hydrogen atoms were added. Different orientation of the lead molecules concerning the target protein was evaluated by the Autodock program and the best dock pose was selected based on the interaction study analysis.

Figure 1: Protein Tyrosinase (PDB) - 1WX3 - Structure in 3D



Ligand preparation

The herbs were selected as per the Siddha classical textbook *Gunapadam mooligai vaguppu* with the indication for treating skin problems like *venkuttam and leprosy*. The following 9 herbs were included by reviewing the previous research articles in Table.1 which had showed their bio-active molecules (7-15) such as Kaempferol from *Indigofera aspalathoides* (Vahl.ex.DC) *(Sivanar vembu),* Quercetin from *Smilax china* (Linn.) *(Parangipattai),* Tinosporide from *Tinospora cordifolia* (Willd.) Hook.f.&Thoms. *(Seenthil),* Anacardic acid from *Semecarpus anacardium* (Linn.) *(Seraangkottai),* Glabridin from *Glycyrrhiza glabra* (Linn.) *(Athimathuram),* Isovitexin from *Psoralea corylifolia* (Linn.) *(Karbogarisi),* Oleic acid from *Nigella sativa* (Linn.) *(Karungjeerakam),* Aristolochic acid from *Aristolochia bracteolata* (Lam.) *(Aadutheendapalai)* & Hydnocarpin from *Hydnocarpus laurifolia* (Dennst.) Sleumer *(Neeradimuthu).* They were retrieved from a systematic literature review and IMPAAT database.

Methodology

Docking calculations were carried out for retrieved bio-active compounds Kaempferol from

Indigofera aspalathoides (Vahl.ex.DC), Quercetin from *Smilax china* (Linn.), Tinosporide from *Tinospora cordifolia* (Willd.) Hook.f&Thoms, Anacardic acid from *Semecarpus anacardium* (Linn.), Glabridin from *Glycyrrhiza glabra* (Linn.), Isovitexin from *Psoralea corylifolia* (Linn.), Oleic acid from *Nigella sativa* (Linn.), Aristolochic acid from *Aristolochia bracteolata* (Lam.) & Hydnocarpin from *Hydnocarpus laurifolia* (Dennst.) Sleumer. against the target protein Tyrosinase(PDB)-1WX3 using Auto Dock 4 software. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined.

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 60×60×60 Å grid points and 0.375 Å spacing were generated using the Autogrid program. 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. The 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 A, and quaternion and torsion steps of 5 were applied (16, 17,18).

Table 1: List of phytocomponents used in the selected herbs for molecular docking(7-15)

Sl. No	Botanical Name	Tamil Name(19)	Selected Phyto-chemicals
1	*Indigofera aspalathoides* (Vahl ex. DC)	*Sivanar vembu*	Kaempferol
2	*Smilax china*(Linn.)	*Parangipattai*	Quercetin
3	*Tinospora cordifolia*(Willd.) Hook.f. and Thoms.	*Seenthil*	Tinosporide
4	*Semecarpus anacardium*(Linn.)	*Seraang kottai*	Anacardic acid
5	*Glycyrrhiza glabra*(Linn.)	*Athimathuram*	Glabridin
6	*Psoralea corylifolia*(Linn.)	*Karboga arisi*	Isovitexin
7	*Nigella sativa*(Linn.)	*Karunjjeerakam*	Oleic acid
8	*Aristolochia bracteolata*(Lam.)	*Aadutheenda palai*	Aristolochic acid
9	*Hydnocarpus laurifolia* (Dennst.) Sleumer	*Neeradi muthu*	Hydnocarpin

Fig.2. Structure of Selected Ligands in 2D and 3D

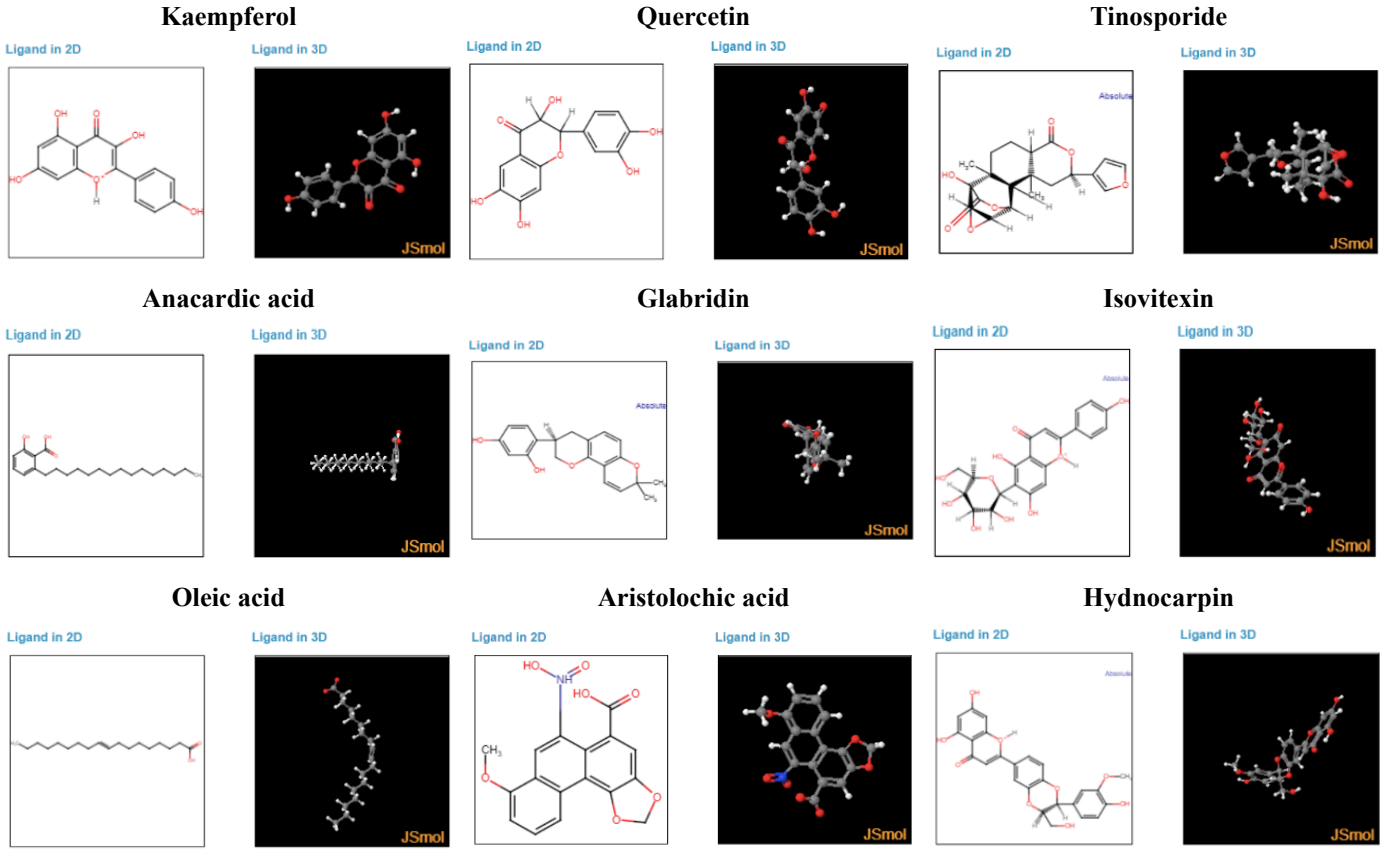


Figure 3. Docking Poses, Plot analysis by 2D interaction and core amino acid analysis by Hydrogen bond plotting of all the bioactive molecules of the selected herbs against the target Tyrosinase (PDB) - 1WX3

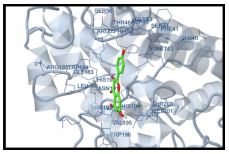
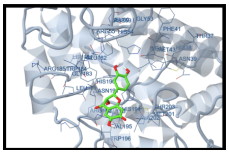
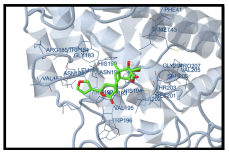
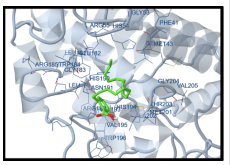
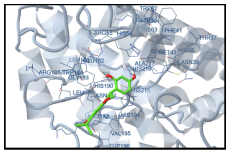
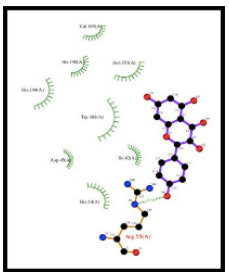
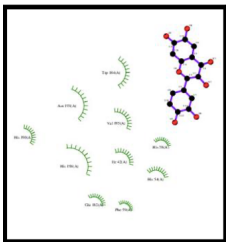
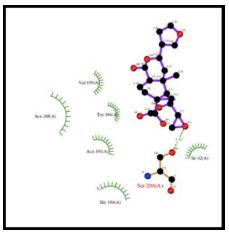
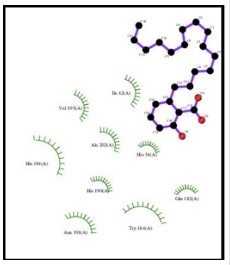
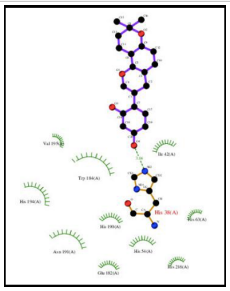
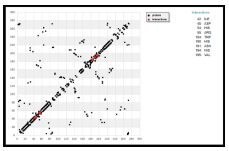
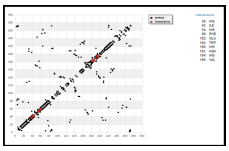
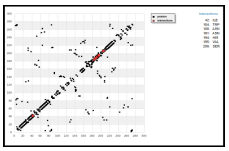
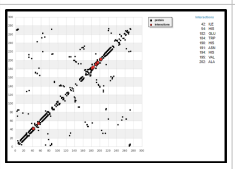
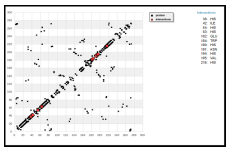
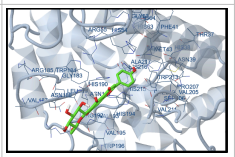
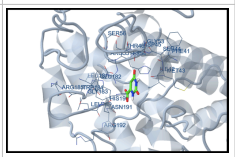
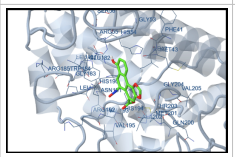
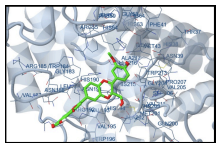
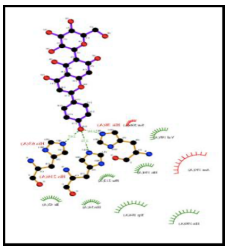
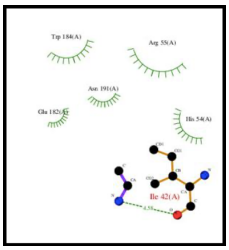
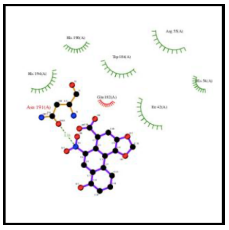
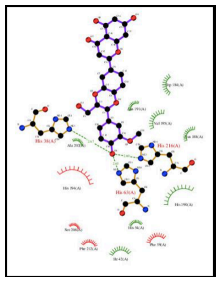
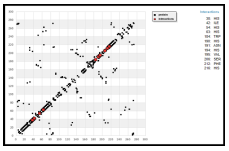
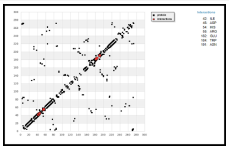
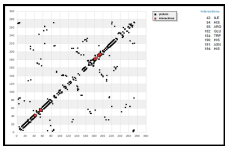
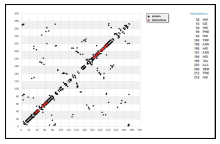
	Kaempferol with Tyrosinase (1WX3)	Quercetin with Tyrosinase (1WX3)	Tinosporide with Tyrosinase (1WX3)	Anacardic acid with Tyrosinase (1WX3)	Glabradin with Tyrosinase (1WX3)
Docking Pose					
Plot analysis by 2D interaction					
Core amino acid Analysis by Hydrogen bond plotting					
	Isovitexin with Tyrosinase (1WX3)	**Oleic acid with Tyrosinase (1WX3)**	**Aristolochic acid with Tyrosinase (1WX3)**	**Hydnocarpin with Tyrosinase (1WX3)**	
Docking Pose					
Plot analysis by 2D interaction					
Core amino acid Analysis by Hydrogen bond plotting					

Table 2: Selected Compounds for the Docking and its Ligand Properties

Bioactive Components	Molar mass g/mol	Molecular Formula	Donor of H Bond	Acceptor of H Bond	Rotational bonds
Kaempferol	286.239	C15H10O6	4	6	1
Quercetin	302.23	C15H10O7	5	7	1
Tinosporide	374.4	C20H22O7	1	7	1
Anacardic acid	348.5	C22H36O3	2	3	15
Glabridin	324.4	C20H20O4	2	4	1
Isovitexin	432.4	C21H20O10	7	10	3
Oleic acid	282.5	C18H34O2	1	2	15

Aristolochic acid	341.27	C17H11NO7	1	7	2
Hydnocarpin	464.4	C25H20O9	4	9	4

Table 3: Bioactive components against Tyrosinase (PDB) - 1WX3 - Summary

Bioactive Components	Est. Free Energy of Binding (kcal/mol)	Est. Inhibition Constant, Ki	Energy of Electrostatics (kcal/mol)	Intermolecular Energy (Total) (kcal/mol)	Interaction Surface
Kaempferol	-4.49	510.33 uM	-0.22	-4.91	590.49
Quercetin	-6.25	26.13 uM	-0.06	-5.53	595.214
Tinosporide	-5.64	73.55 uM	-0.14	-6.22	653.101
Anacardic acid	-5.89	47.90 uM	-0.09	-7.88	677.064
Glabridin	-4.72	346.77 uM	-0.02	-5.58	612.505
Isovitexin	-2.42	16.69 mM	-0.12	-2.72	661.536
Oleic acid	-3.17	4.74 mM	-0.13	-3.47	357.641
Aristolochic acid	-5.29	131.81 uM	-0.27	-6.22	608.598
Hydnocarpin	-4.87	268.38 uM	-0.06	-5.78	594.761

Table 4: Bioactive components with amino acid residue interactions of Tyrosinase

Bioactive Components	Number of Interactions	Aminoacid Residues													
Kaempferol	3	42 ILE	45 ASP	54 HIS	55 ARG	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL					
Quercetin	4	38 HIS	42 ILE	54 HIS	59 PHE	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL				
Tinosporide	1	42 ILE	184 TRP	188 ASN	191 ASN	194 HIS	195 VAL	206 SER							
Anacardic acid	3	42 ILE	54 HIS	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	202 ALA					
Glabridin	4	38 HIS	42 ILE	54 HIS	63 HIS	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	216 HIS			
Isovitexin	4	38 HIS	42 ILE	54 HIS	63 HIS	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	206 SER	212 PHE	216 HIS		
Oleic acid	1	42 ILE	45 ASP	54 HIS	55 ARG	182 GLU	184 TRP	191 ASN							
Aristolochic acid	2	42 ILE	45 ASP	55 ARG	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS						
Hydnocarpin	4	38 HIS	42 ILE	54 HIS	59 PHE	63 HIS	184 TRP	188 ASN	190 HIS	191 ASN	194 HIS	195 VAL	206 SER	212 PHE	216 HIS

Results and Discussion

Current therapeutic management available for vitiligo is only moderately effective in controlling further severity of symptoms. Hence in recent times, people rely most on alternative complementary treatments for the management of vitiligo. In addition to having vitiligo, 15 to 25% of those suffering from it also have at least one other autoimmune disorder, particularly autoimmune thyroid disease, rheumatoid arthritis, type 1 diabetes, psoriasis, pernicious anaemia, Addison disease, systemic lupus erythematosis, celiac disease, Crohn's disease or ulcerative colitis (20).

A total of 9 bioactive lead compounds present in the herbs were selected with the help of previously published research articles which are primarily used in the treatment of skin disorders like vitiligo, leprosy etc., in the Siddha system of medicine. From the reported data of the herbs, it was found that Quercetin showed the highest binding affinity of -6.25 kcal/mol. Then, Anacardic acid showed the second highest binding affinity of -5.89 kcal/mol to the amino acids His 54, His 190 & His 194, followed by Tinosporide, Aristolochic acid, Hydnocarpin, Glabridin, Kaempferol, Oleic acid and Isovitexin with binding energies of -5.64 kcal/mol, -5.29 kcal/mol, -4.87 kcal/mol, -4.72 kcal/mol, -4.49 kcal/mol, -3.17 kcal/mol, -2.42 kcal/mol respectively (Table 3).

Quercetin, Glabridin, Isovitexin, Hydnocarpin share four active site amino-acid in common such as 38 HIS, 54 HIS, 190 HIS & 194 HIS and Kaempferol, Quercetin, Anacardic acid, Glabridin, Isovitexin and Hydnocarpin shared 3 active site amino-acid such as 54 HIS, 190 HIS & 194 HIS in common. Tinosporide (194 HIS) and Oleic acid (54 HIS) interact with only one active site amino acid (Table 4). Though the compound Quercetin has the highest binding energy among all the compounds, while considering the interactions, it showed 4 interactions with the amino-acid residues. From the reported data of the herbs, the leads such as Quercetin, Glabridin, Isovitexin and Hydnocarpin possess four interactions with 70-100% binding efficacy by interacting with core target amino acids (His 38, His54, His190, His194) present on the protein – Tyrosinase enzyme.

Conclusion

Based on the findings of the computational analysis, it was determined that the bioactive compounds found in the selected herbs, such as Quercetin, Anacardic Acid, Glabridin, Isovitexin and Hydnocarpin, reveal significant binding against the target protein by interacting with amino-acid present on the active site of the tyrosinase enzyme, thereby it was determined that these compounds may exert promising anti-vitiligo property. It is necessary to conduct additional preclinical and clinical trials to determine the precise mechanism and effectiveness of the chosen herbs in the treatment of vitiligo.

Conflict of Interest

The authors hereby declare that they have no conflicts of interest to disclose.

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