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ANTI-VITILIGO ACTIVITY OF LEAD COMPOUNDS OF KARPOGI PASAI TARGET PROTEIN TYROSINASE -A MOLECULAR DOCKING STUDY

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Abstract

Around 50 million people in the world are affected by Vitiligo which is a chronic inflammatory cutaneous disorder. Vitiligo is not a life-threatening disease, it causes disfigurement in the appearance leading to psychological harm to patients. Moreover, vitiligo favours vulnerability to other diseases like arthritis, hyperthyroid's, diabetes and lupus erythematosus. Siddha system is a traditional system that has originated in India since 4000 years ago with unique treatment methods and herbomineral formulations for various skin diseases and autoimmune diseases. The present study is aimed to execute the in-Silico computational studies of phytoconstituents of Siddha formulation Karbogi pasai that has been indicated for the treatment of vitiligo(Venpadai).The study was aimed to find the lead molecules in the herb Karbogi pasai a Siddha formulation to bind with specific amino acid residues in the tyrosinase enzyme, which helps in melanogenesis, to enhance tyrosinase activity and improve hypopigmentation medical conditions like vitiligo. Docking analysis of 11 phytoconstituents in Karbogi pasai was performed using AutoDock program. The lead compound Isovitexin was found to have the strongest binding affinity against tyrosinase. Bavachinin had the lowest Ki value and Pantothenic acid had the strongest electrostatic interaction. Overall, the study found potential antivitiligo effects of the herb's phytoconstituents.

Keywords: Vitiligo, Karbogi pasai, Tyrosinase, Venpadai, Skin disease

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1. Introduction

Vitiligo is a depigmentation disorder of the skin and hair follicles characterized by the destruction of the melanocytes, mainly exhibiting clinical manifestation as expanding hypopigmented lesions of the skin.[1] Clinically, vitiligo is divided into the most common non-segmental vitiligo, segmental vitiligo and mixed vitiligo.[2] The aetiology of vitiligo is multifactorial and not yet fully understood, but it is generally believed to be an autoimmune-mediated process, resulting from the destruction of melanocytes by the body's own immune system. [3][4] Previous studies have indicated that systemic oxidative stress could be considered as one of the important pathogenic events in melanocyte loss in vitiligo patients.[5][6] Oxidative stress may induce and mediate multiple genes encoding proteins through several signalling pathways, including tyrosinase (TYR), microphthalmia-associated transcription factor (MITF), MAPK, Nrf2-ARE and ultimately leads to melanin loss and vitiligo.[7] Apart from the autoimmune and oxidative stress, neural and biochemical causes are also proposed for the cause of vitiligo.[8]

Research on treating vitiligo with small molecules including natural products [9] and derivatives, anti-oxidative stress drugs, immunosuppressants.[10] Natural compounds have recently shown a wide range of therapeutic bioactivities against a number of skin disorders.[11] Natural products have been demonstrated to enhance melanin production with UVB target phototherapy and inhibit its degradation in a coordinated manner through in vivo and in vitro experiments.[12] Vitiligo treatment methods rely on multitude of factors like scavenging free radicals, such as NOS, mitigating oxidative stress-induced damage to melanocytes, activating melanogenesis-related signalling pathways, upregulating the expression of tyrosinase gene, downregulating the expression of chemokines and inflammatory cytokines.[13]

There have been several studies conducted on herbal compositions for the treatment of vitiligo. A study published in the Journal of Ethnopharmacology in 2004 found that a combination of Psoralea corylifolia (Babchi) and Tamarindus indica (Imli) showed significant repigmentation in patients with vitiligo.[14] Similarly, a study published in the Journal of Clinical and Aesthetic Dermatology in 2015 found that a combination of curcumin and psoralen showed significant improvement in patients with vitiligo.[15] A study published in the Journal of Dermatological Treatment in 2014 found that a topical application of Ginkgo biloba extract showed significant improvement in patients with vitiligo.[16] Apart from herbal extract combinations, studies on single natural compounds were also found to be effective against vitiligo. Piperine, a compound found in Piper nigrum that has been shown to have anti-inflammatory and immunomodulatory properties showed significant repigmentation in patients with vitiligo after a topical application of piperine.[17] Caffeine, an alkaloid present in many plant species including Coffea arabica and Coffea canephora can be used as an anti-vitiligo treatment.[18] Commonly, wild seeds of Vernohia anthelmintica are known to exhibit a significant effect in the treatment of melanin deficiency and vitiligo [19] from which butin an potent antivitiligo component was isolated.[20] Medicinal plants like Phlebodium aureum, Cullen corylifolium, Picrorhiza kurroa, and Baccharoides anthelmintica are proposed to be having antivitiligo properties.[21] "Karbogi pasai" an Indian traditional medicine prescribed to treat skin disorders is a combination of medicinal herbs such as Terminalia chebula, Terminalia bellerica, Emblica officinalis, Acacia catechu, Pterocarpus marsupium, Psoralea corylifolia mixed with prepared medicines such as aya chendooram, navapasana chendooram, naga parpam, thamira chendooram at different concentrations. In this study, "Karbogi pasai" is studied for antivitiligo tendency through docking analysis of its phytoconstituents. The principal objective of the investigation is to identify lead compounds that can bind to the core bioactive amino acid residues of the tyrosinase enzyme, namely His38, His54, His63, His190, His194, and His216. These amino acids are known to facilitate the enzymatic activity of tyrosinase and are therefore believed to enhance or synergize its action, thereby promoting melanogenesis. The improvement of melanin production through increased tyrosinase activity is critical to achieving melanogenesis, which is commonly impaired in medical conditions characterized by hypopigmentation, such as vitiligo.

2. Materials and Methods

Crystalline structure of the target protein Tyrosinase (PDB ID: 1WX3) was retrieved from protein data bank (Figure 1) and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules Isovitexin, Bavachinin, Hydnocarpin, Pantothenic acid, Biotin, Morphine, Thebaine, Abscisic acid, Limonene, Thymol and Nigellamine (Figure 2) with respect to the target protein was evaluated by AutoDock program and the best dock pose was selected based on the interaction study analysis. Docking calculations were carried out using Auto Dock 4 for the retrieved phytocomponents against the target

protein Tyrosinase (PDB ID: 1WX3). 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.[22] Å grid points and 0.375 Å spacing were generated using the Autogrid program.[22] 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.[23] 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.

3. Results and Discussion

Total of 11 bioactive lead compounds (Table 1) were retrieved from the herbs present in the siddha formulation Karbogi pasai. Isovitexin has the most negative free energy of binding (-4.20 kcal/mol), suggesting that it has the strongest binding affinity among all the compounds. Isovitexin is known to have anti-inflammatory and anti-oxidant activities through reduction in iNOS, cyclooxygenase-2 (COX-2), MAPK expression in acute lung injury induced by lipopolysaccharides. [24] Bavachinin has the lowest Ki value (25.22 μM), Pantothenic Acid has the lowest electrostatic energy (-0.57 kcal/mol) and the largest interaction surface (724.876 Å²) indicating that it has the strongest electrostatic interaction with a high degree of contact with the target protein (Table 2). Bavachinin is a recently studied Pan-peroxisome proliferator-activated receptor (PPAR) agonist which could have glucose and lipid-lowering effect confirmed through mouse model. [25] It is also known for monoamine oxidase B inhibitory activity; inhibitors which are commonly used in the early management of Parkinson's disease.[26] Isovitexin and Bavachinin are phytoconstituents commonly found in Psoralea corylifolia [27] used in many Asian traditional medicines. From reported data of the herb, the leads such as Limonene, Bavachinin, Isovitexin, Abscisic Acid, Thymol, Pantothenic Acid and Thebaine possess 4-6 interactions with 60-100% binding efficacy by interacting with core target amino acids (His38, His54, His63, His190, His194 and His216) present on the protein – Tyrosinase enzyme followed by which the compounds such as Biotin, Hydnocarpin, Morphine and Nigellamine possess 2-4 interactions which accounts for 30-50% binding efficacy by interacting with target amino acids on the enzyme Tyrosinase. The His54 is only amino acid that interacts with all of the compounds. Additionally, there are four amino acids that interact with nine out of the eleven compounds, namely His38, His190, Ile42, and Asn191 (Table 3). Approximately 37 natural substances are recognized to enhance melanin synthesis by upregulating tyrosinase activity in a melanocyte model in vitro. However, their in vivo potential for mitigating autoimmune destruction remains unexplored.[28]

Figure 1: 3D structure of Tyrosinase (PDB ID: 1WX3) used as the target protein in molecular docking

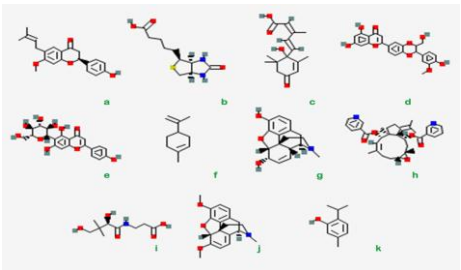

Figure 2: 2D structures of the selected ligands a. Bavachinin, b. Biotin, c. Dormin, d. Hydnocarpin, e. Isovitexin, f. Limonene, g. Morphine, h. Nigellamine, i. Pantothenic Acid, j. Thebaine, k. Thymol.

Table 1: 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
Limonene	136.23	$C_{10}H_{16}$	0	0	1
Bavachinin	338.4	$C_{21}H_{22}O_4$	1	4	4
Biotin	244.31	$C_{10}H_{16}N_2O_3S$	3	4	5
Hydnocarpin	464.4	$C_{25}H_{20}O_9$	4	9	4
Isovitexin	432.4	$C_{21}H_{20}O_{10}$	7	10	3
Abscisic Acid	264.32	$C_{15}H_{20}O_4$	2	4	3
Morphine	285.34	$C_{17}H_{19}NO_3$	2	4	0
Nigellamine	530.7	$C_{32}H_{38}N_2O_5$	0	7	6
Thymol	150.221	$C_{10}H_{14}O$	1	1	1
Pantothenic Acid	219.23	$C_9H_{17}NO_5$	4	5	6
Thebaine	311.4	$C_{19}H_{21}NO_3$	0	4	2

Table 2: Summary of the molecular docking studies of compounds against Tyrosinase (1WX3)

Compound	Est. Free Energy of Binding (kcal/mol)	Est. Inhibition Constant, Ki (uM)	Electrostatic Energy (kcal/mol)	Total Intermolec. Energy (kcal/mol)	Interact. Surface
Limonene	-4.65	387.26	-0.01	-4.96	444.326
Bavachinin	-6.27	25.22	-0.06	-7.54	721.631
Biotin	-6.04	37.35	-0.13	-6.13	494.255
Hydnocarpin	-4.68	369.29	-0.56	-6.91	700.602
Isovitexin	-4.20	828.38	-0.19	-3.70	755.568
Abscisic Acid	-6.15	31.21	-0.17	-6.99	608.639
Morphine	-5.44	102.69	-0.02	-6.42	572.291
Nigellamine	-5.43	104.94	-0.19	-6.58	584.273
Thymol	-6.23	27.06	-0.52	-8.38	689.202
Pantothenic Acid	-5.65	71.61	-0.57	-7.56	724.876
Thebaine	-5.52	90.33	-0.21	-7.29	694.492

Table 3: Amino acid residue interaction of lead and standard against crystal structure of tyrosinase (1WX3)

Compounds	Interactions	Amino acid residues											
Limonene	5	38 HIS	42 ILE	54 HIS	190 HIS	194 HIS	206 SER	216 HIS					
Bavachinin	6	38 HIS	42 ILE	43 MET	54 HIS	55 ARG	59 PHE	63 HIS	184 TRP	190 HIS	194 HIS	206 SER	216 HIS

Biotin	3	38 HIS	42 ILE	190 HIS	191 ASN	194 HIS	195 VAL	202 ALA	203 THR	206 SER			
Hydnocarpin	2	94 TRP	165 ARG	168 VAL	169 LEU	171 ALA	172 LEU	190 HIS	194 HIS	203 LEU	236 ARG	237 ILE	240 VAL
Isovitexin	6	38 HIS	42 ILE	54 HIS	63 HIS	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	206 SER	212 PHE	216 HIS
Abscisic Acid	5	38 HIS	42 ILE	54 HIS	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	202 ALA	206 SER	212 PHE	216 HIS
Morphine	3	42 ILE	54 HIS	55 ARG	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	203 THR	206 SER	
Nigellamine	3	42 ILE	45 ASP	54 HIS	55 ARG	184 TRP	190 HIS	194 HIS					
Thymol	4	38 HIS	42 ILE	54 HIS	190 HIS	191 ASN	194 HIS	206 SER					
Pantothenic Acid	4	38 HIS	42 ILE	54 HIS	55 ARG	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	206 SER		
Thebaine	5	38 HIS	42 ILE	54 HIS	55 ARG	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	206 SER	212 PHE	216 HIS

4. Conclusion

Based on the results of the computational analysis, it was concluded that the bio-active compounds like Limonene, Bavachinin, Isovitexin, Abscisic Acid, Thymol, Pantothenic Acid, Thebaine, Biotin, Hydnocarpin, Morphine and Nigellamine present in the Siddha formulation "Karbogi pasai" reveal significant binding against the target protein by interacting with amino acids present on the active site of the tyrosinase enzyme. So, it is safe to assume that these compounds exert promising anti-vitiligo property by synergizing the action of tyrosinase enzyme to improve melanogenesis. This treatment can improve melanin pigment production which is actually found to be deprived in vitiligo. Plant based natural products with melanin expression inducing capabilities are becoming a promising route to treat vitiligo in future.

5. References

1. Buggiani G, Tsampau D, Hercogovà J, et al. Clinical efficacy of a novel topical formulation for vitiligo:compared evaluation of different treatment modalities in 149 patients. Dermatol Ther. 2012;25(5):472–6. https://doi.org/10.1111/j.1529-8019.2012.01484.x PMid:23046028.
2. Cong-Cong Li. Phytochemical and Pharmacological Studies on the Genus Psoralea: A Mini Review. Evidence-Based Complementary and Alternative Medicine .2016. https://doi.org/10.1155/2016/8108643.
3. Dell'Anna, M. L., Mastrofrancesco, A., Sala, R., Venturini, M., Ottaviani, M., Vidolin, A. P., et al. (2007). Antioxidants and Narrow Band-UVB in the Treatment of Vitiligo: a Double-Blind Placebo Controlled Trial. Clin. Exp. Dermatol. 32 (6), 631–636. doi:10.1111/j.1365-2230.2007.02514.x.
4. Gianfaldoni S, Wollina U, Tirant M, Tchernev G, Lotti J, Satolli F, Rovesti M, França K, Lotti T. Herbal Compounds for the Treatment of Vitiligo: A Review. Open Access Maced J Med Sci. 2018 Jan 21;6(1):203-207. doi: 10.3889/oamjms.2018.048. PMID: 29484024; PMCID: PMC5816300.
5. Speeckaert, R., Dugardin, J., Lambert, J., Lapeere, H., Verhaeghe, E., Speeckaert, M. M., et al. (2018). Critical Appraisal of the Oxidative Stress Pathway in Vitiligo: a Systematic Review and Meta-Analysis. J. Eur.

Acad. Dermatol. Venereol. 32 (7), 1089–1098. doi:10.1111/jdv.14792.
6. Wu Heng, Niu Chao and Aisa Haji Akber, Research Progress of Small Molecules as Anti-vitiligo Agents, Current Medicinal Chemistry 2023; 30. https://dx.doi.org/10.2174/0929867330666230214103054
7. Zarmouh NO, Mazzio EA, Elshami FM, Messeha SS, Eyunni SV, Soliman KF. Evaluation of the Inhibitory Effects of Bavachinin and Bavachin on Human Monoamine Oxidases A and B. Evid Based Complement Alternat Med. 2015;2015:852194. doi: 10.1155/2015/852194. Epub 2015 Oct 19. PMID: 26557867; PMCID: PMC4629031.
8. Feng L, Luo H, Xu Z, Yang Z, Du G, Zhang Y, Yu L, Hu K, Zhu W, Tong Q, Chen K, Guo F, Huang C, Li Y. Bavachinin, as a novel natural pan-PPAR agonist, exhibits unique synergistic effects with synthetic PPAR-γ and PPAR-α agonists on carbohydrate and lipid metabolism in db/db and diet-induced obese mice. Diabetologia. 2016 Jun;59(6):1276-86. doi: 10.1007/s00125-016-3912-9. Epub 2016 Mar 16. PMID: 26983922.
9. D.R. Shi, L.S. Ha, X. Ru, H.F. Gan, J.L. Liu, X.M. Pu, Clinical efficacy of treatment of Vernonia anthelmintica (L.) Willd on vitiligo, Xinjiang Med. J. 33 (2003) 51–52.
10. Z.Q. Ma, H. Hu, T.T. He, H. Guo, M.Y. Zhang, M.W. Chen, Y.T. Wang, An assessment of traditional Uighur medicine in current Xinjiang region (China), Afr. J. Tradit. Complem. 11 (2014) 301–314.
11. S.X. Huo, Q. Wang, X.M. Liu, C.H. Ge, L. Gao, X.M. Peng, M. Yan, The effect of butin on the vitiligo mouse model induced by hydroquinone, Phytother. Res. 31 (2017) 740–746.
12. Gohil, K. J., Patel, J. A., & Gajjar, A. K. (2004). Pharmacological Review on Natural Medicinal Plant Used as Anti Vitiligo Agent. Journal of Ethnopharmacology, 104(1-2), 68-75. https://doi.org/10.1016/j.jep.2005.08.059.
13. Parsad, D., Pandhi, R., & Juneja, A. (2014). Effectiveness of Ginkgo biloba in treating vitiligo: A randomized controlled trial. Journal of Dermatological Treatment, 25(2), 115-117. https://doi.org/10.3109/09546634.2013.768328.
14. Rashidi, T., Mahroo, V., & Yaghmaei, P. (2013). Topical Piperine for the Treatment of Vitiligo. Journal of Dermatological Science, 72(3), 287-290. https://doi.org/10.1016/j.jdermsci.2013.07.004.
15. Handa, S., Kaur, I., & Sharma, V. K. (2015). Comparative Efficacy of Combination of Curcumin and Psoralen with Ultraviolet-A and Narrowband Ultraviolet-B in Psoralen and Ultraviolet-A Therapy for Vitiligo. Journal of Clinical and Aesthetic Dermatology, 8(9), 43-47. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4568143.
16. E. Nicolaidou, A.D. Katsambas, Pigmentation disorders: hyperpigmentation and hypo-pigmentation, Clin. Dermatol. 32 (2014) 66–72.
17. N. Malhotra, M. Dytoc, The pathogenesis of vitiligo, J. Cutan. Med. Surg. 17 (2013) 153–172.
18. I.C.L. Poole, P.K. Das, R.M.J.G.J. van den Wijngaard, J.D. Bos, W. Westerhof, Review of the etiopathomechanism of vitiligo: a convergence theory, Exp. Dermatol. 2 (1993), 145–153
19. Ezzedine, K., Eleftheriadou, V., Whitton, M., and van Geel, N. (2015). Vitiligo. The Lancet 386 (9988), 74–84. doi:10.1016/s0140-6736(14)60763-7.
20. Feily A, Saboktakin M. Caffeine as a novel addition to the antivitiligo ammunition. G Ital Dermatol Venereol. 2010 Feb;145(1):139. PMID: 20197754.
21. A.A. Shah, A.A. Sinha, Oxidative stress and autoimmune skin disease, Eur. J. Dermatol. 23 (2013) 5–13.
22. L. Guerra, E. Dellambra, S. Brescia, D. Raskovic, Vitiligo: pathogenetic hypotheses and targets for current therapies, Curr. Drug. Metab. 11 (2010) 451–467.
23. Slominski, D.J. Tobin, S. Shibahara, J. Wortsman, Melanin pigmentation in mammalian skin and its hormonal regulation, Physiolo. Rev. 84 (2004) 1155–1228.
24. Jian, Z., Li, K., Liu, L., Zhang, Y., Zhou, Z., Li, C., et al. (2011). Heme Oxygenase-1 Protects Human Melanocytes from H2O2-Induced Oxidative Stress via the Nrf2-ARE Pathway. J. Invest. Dermatol. 131 (7), 1420–1427. doi:10.1038/jid.2011.56.
25. Pang Y, Wu S, He Y, Nian Q, Lei J, Yao Y, Guo J and Zeng J (2021) Plant-Derived Compounds as Promising Therapeutics for Vitiligo. Front. Pharmacol. 12:685116. doi: 10.3389/fphar.2021.685116.
26. Castillo E, González-Rosende ME, Martínez-Solís I. The Use of Herbal Medicine in the Treatment of Vitiligo: An Updated Review. Planta Med. 2022 Nov 15. doi: 10.1055/a-1855-1839. Epub ahead of print. PMID: 36379447.
27. Qadir A, Ullah SNMN, Jahan S, Ali A, Khan N. Drug delivery of natural products through nano-carriers for effective vitiligo therapy: A compendia review. J Cosmet Dermatol. 2022 Nov;21(11):5386-5404. doi:

10.1111/jocd.15158. Epub 2022 Jun 25. PMID: 35699364.

28. Lv, H., Yu, Z., Zheng, Y., Wang, L., Qin, X., Cheng, G., & Ci, X. (2016). Isovitexin Exerts Anti-Inflammatory and Anti-Oxidant Activities on Lipopolysaccharide-Induced Acute Lung Injury by Inhibiting MAPK and NF-κB and Activating HO-1/Nrf2 Pathways. International Journal of Biological Sciences, 12, 72 - 86.
29. Yin, L., Niu, C., Liao, L. X., Dou, J., Habasi, M., and Aisa, H. A. (2017). An Isoxazole Chalcone Derivative Enhances Melanogenesis in B16 Melanoma Cells via the Akt/GSK3β/β-Catenin Signaling Pathways. Molecules 22 (12), 2077. doi:10.3390/molecules22122077.