Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/mv8b6y44

HARNESSING TYROSINASE ACTIVATION FOR VITILIGO INTERVENTION: AN IN SILICO STUDY OF SIVAKARANDHAI KARPAM FROM SIDDHA MEDICINE

A Kalaiarasi[1*], S M Chitra[2]

[1*]PG Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India. Email: kalaiarasiangappan0@gmail.com
[2]Assistant professor, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India. Email: Chittu758@gmail.com

***Corresponding Author:** Kalaiarasi Angappan
*PG Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India. Email: kalaiarasiangappan0@gmail.com

Abstract

Introduction:

Vitiligo is a chronic skin disorder characterized by the progressive destruction of melanocytes, resulting in depigmented patches on the skin. Tyrosinase, a key enzyme in the melanogenesis pathway plays a central role in melanin synthesis and is considered a critical target for therapeutic intervention in vitiligo.

Materials and Methods:

Sivakarandai (Sphaeranthus amaranthoides), a medicinal plant renowned for its diverse pharmacological properties and traditionally used as a *kaya karpam* (rejuvenation therapy) in Siddha medicine, was investigated in this study for its potential anti-vitiligo activity using in silico approaches. Phytochemicals from *Sphaeranthus amaranthoides* were retrieved and subjected to molecular docking to assess their binding affinity towards the tyrosinase enzyme. In-silico docking simulations were performed using Auto Dock v4, followed by interaction visualization in drug discovery Studio, to evaluate the binding efficacy of 4 bioactive compounds with the target protein tyrosinase.

Results:

Among the screened compounds, Chrysosplenol, Palmitic acid, Linoleic acid, and Squalene demonstrated notable docking scores ranging from -4.83 to -7.28 kcal/mol and formed three to four interactions with active site residues. Squalene exhibited the highest binding affinity, while Chrysosplenol, Palmitic acid shared four common active site interactions with the tyrosinase enzyme. Linoleic acid shared three active site residues.

Conclusion:

The study highlights that these bioactive compounds exhibit favorable binding affinities and stable interactions within the tyrosinase active site, suggesting their potential for novel anti-vitiligo properties. Further, in vitro and in vivo studies are warranted to validate these findings and explore their therapeutic efficacy**.**

Keywords: Venpadai, Vitiligo, Sivakarandhai karpam,Siddha medicine,Tyrosinase.

Introduction

Vitiligo is a skin condition that develops over time and is marked by the gradual disappearance of melanocytes, the cells that generate the pigment melanin[1]. The unpredictable nature of the condition—where lesions may either spread quickly or remain stable for many years[1,5]. Research based on population data estimates that the worldwide occurrence of vitiligo varies from 0.1% to 2%, with certain studies indicating rates reaching up to 8% [2,3,4]. Although it does not pose a threat to life, it greatly impacts an individual's quality of life by causing emotional distress, social discrimination, and feelings of isolation[1,5]. Vitiligo is a complex disorder influenced by multiple factors, such as genetic susceptibility, oxidative stress, autoimmune responses and problems with cellular adhesion[6]. Oxidative stress is recognized as a major initiating factor in the development of vitiligo. It encourages the melanocyte-derived self-antigens, promotes the release of damage-associated molecular patterns (DAMPs) and stimulates keratinocytes to secrete chemokines. These changes facilitate the recruitment and activation of CD8+ T cells in the affected skin, resulting in further injury and loss of melanocytes[7].

Siddha medicine, one of India's oldest traditional healing systems, boasts a rich heritage of herbal therapy and natural remedies, originating in South India. The Classical text Siddhar vaithiya Yugimuni perunool-800 referred to skin diseases under the term Kuttam divided it into 18 different categories—including vitiligo, known as "Venpadai", "Venpulli", "Swetha kuttam" or "Venkuttam" in Siddha texts [8] .According to Siddha principles, Venkuttam is classified based on its symptoms and the disturbance of the body's three vital humours (Vatha, Pitha and Kabha) leading to variants such as Vatha Venpadai, Pitha Venpadai, and Kabha Venpadai. Venkuttam typically presents as white patches on the skin that may or may not be thickened along with whitening of body hairs [8,9]. Some individuals also report burning sensation and non-healing ulcers in areas of palm, mouth, lips or genitals [8]. Management of vitiligo in Siddha focuses on a personalized treatment plan that includes both internal medicines and external applications along with recommendations for lifestyle changes and dietary modifications.

Sphaeranthus amaranthoides is a small creeping herb typically found in semi-aquatic habitats and is a member of the Asteraceae family. In contemporary medicine, traditional practices such as Siddha and Ayurveda significantly contribute to drug discovery due to their proven effectiveness and minimal side effects. Siddha medicine makes extensive use of *Sphaeranthus amaranthoides* known as "Sivakaranthai" traditionally administered as chooranam—a kaya karpam (rejuvenate) preparation—particularly for skin disorders such as kuttam. Historically referenced in Bogar Karpam 300, it is also indicated for balancing the humoral doshas (vatham, pitham) when taken over a period for four months [10]. Modern pharmacological studies have validated its broad therapeutic spectrum, noting its antioxidant[11] anti-inflammatory[12] antimicrobial[11] and wound-healing properties [13].
Despite its traditional use and emerging in vitro evidence, the bioactive compounds responsible for its melanogenesis-enhancing effects—potentially through interaction with the tyrosinase enzyme—remain unclear. Tyrosinase, a key enzyme in melanin synthesis, is regulated by core amino acid residues (His38, His54, His63, His190, His194, His216). This study aims to identify the lead molecules in *Sphaeranthus amaranthoides* that may bind these critical residues via molecular docking thereby synergizing tyrosinase activity and elucidating the herb's potential in stimulating melanogenesis for dermatological applications.

Materials and Methods

Preparation of target protein – Tyrosinase (PDB 1WX3)

The crystalline structure of the target protein Tyrosinase (PDB 1WX3) shown in figure 1 was retrieved from the Protein Data Bank, the protein was cleaned up and essential missing hydrogen atoms were added. Different orientations of the lead molecules relative to the target protein were evaluated using the Autodock program, and the best docking pose was selected
based on interaction study analysis.

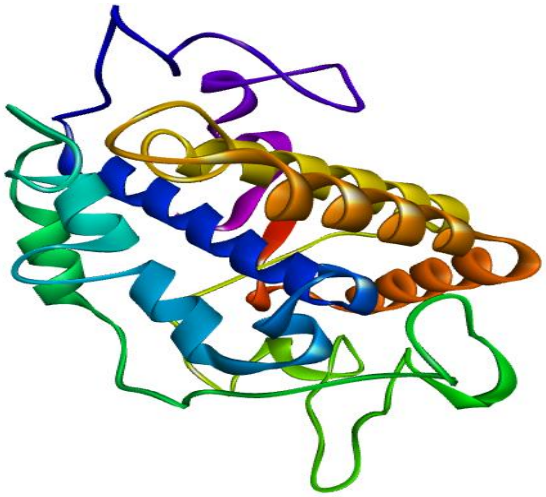


Figure 1: Target Receptor Structure – Tyrosinase (PDB 1WX3)

Ligand preparation for the docking analysis

The little procumbent medicinal herb *Sphaeranthus amaranthoides* is utilized as a restorative treatment for leprosy [10]. From the systemic literature review, the following phytocomponents of *Sphaeranthus amaranthoides*—Chrysosplenol, Linoleic acid, Palmitic acid, and Squalene—were selected for insilco analysis [14-17]. The 2D and 3D structures of the ligand compounds were illustrated in figure 2 and their properties were summarized in Table 1**.**

Methodology

Docking was performed using AutoDock 4. Gasteiger partial charges were assigned to the ligand atoms. Non-polar hydrogens were merged with their attached heavy atoms, and the rotatable bonds in the ligand were identified and defined [18].

Docking studies were conducted for four phytocomponents—Chrysosplenol, Linoleic acid, Palmitic acid, and Squalene—extracted from *Sphaeranthus amaranthoides*, targeting the Tyrosinase protein with PDB ID 1WX3. Essential hydrogen atoms, Kollman united atom charges, and solvation parameters were incorporated using Auto Dock Tools. Grid maps for affinity calculations were created by the Auto grid program, employing a grid with ×× Å points and a spacing of 0.375 Å [20].

The van der Waals interactions were calculated using the Auto Dock parameter set, while distance-dependent dielectric functions were applied for the electrostatic terms. Docking simulations utilized the Lamarckian genetic algorithm (LGA) along with the Solis & Wets local search method [21] .The ligands initial positions, orientations and torsion angles were assigned randomly, with all rotatable bonds permitted to move during docking. Each docking study consisted of two separate runs, each ending after a maximum of 250,000 energy evaluations. The population size for the algorithm was set to 150. The search process used a translational step size of 0.2 Å, while quaternion and torsion step sizes were set to 5 [22,23].

Results

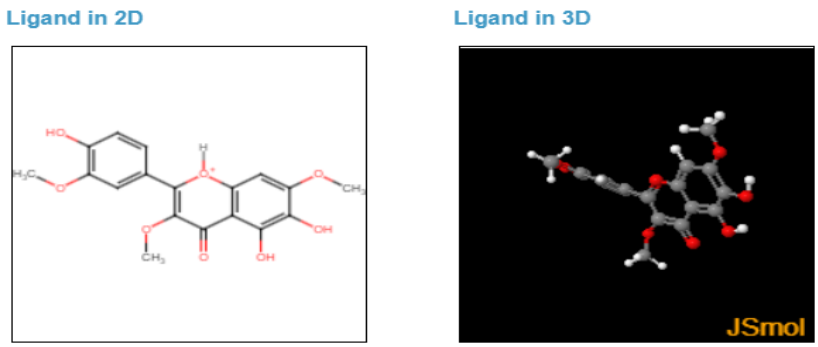
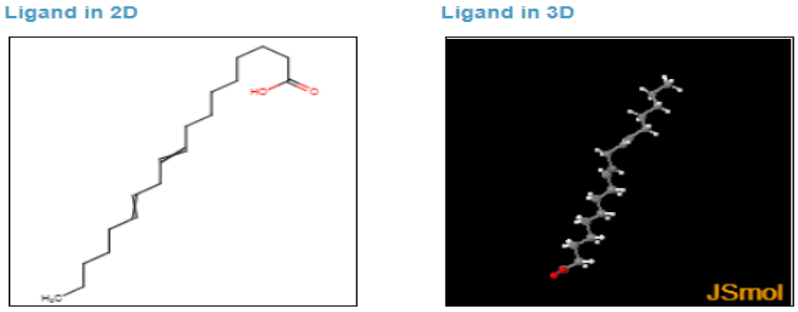
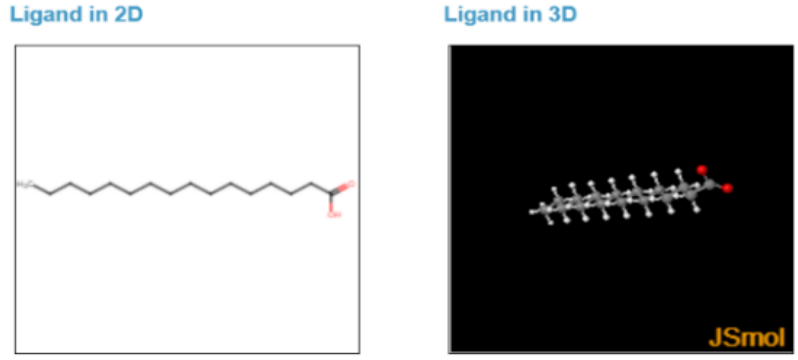
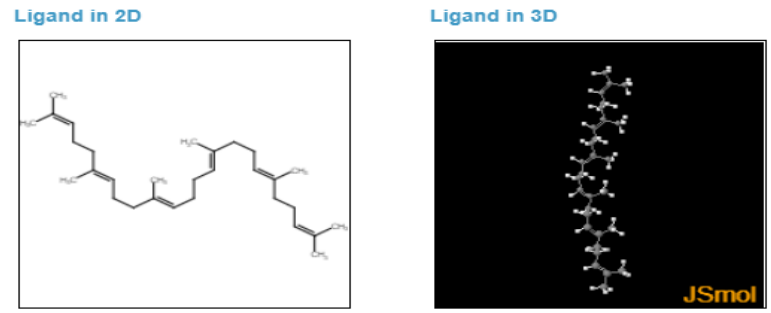
PHYTOCOMPONENTS	STRUCTURES OF 2D AND 3D
Linoleic acid	
Palmitic acid	
Squalene	
Chrysosplenol	

Figure:2 2D and 3D Structure of Phytocomponents

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Chrysosplenol	Linoleic acid	Palmitic acid	Squalene
Molar weight g/mol	360.3 g/mol	280.452 g/mol	256.42 g/mol	410.7 g/mol
Molecular Formula	$C_{18}H_{16}O_8$	$C_{18}H_{32}O_2$	$C_{16}H_{32}O_2$	$C_{30}H_{50}$
H Bond Donor	3	1	1	0
H Bond Acceptor	8	2	2	0
Rotatable bonds	4	14	14	15

g/mol - gram per mole

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

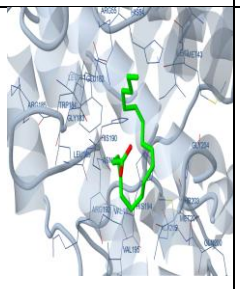
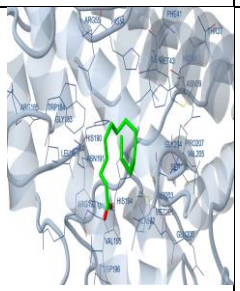
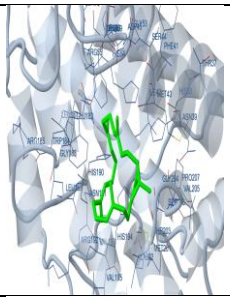
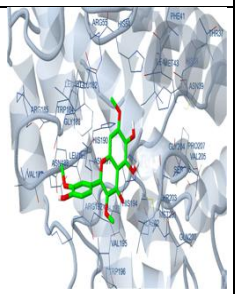
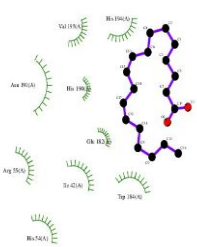
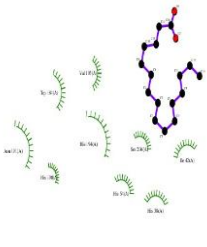
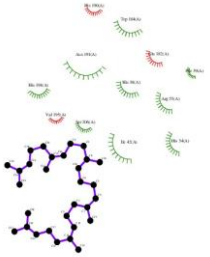
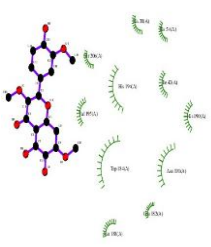
Compounds	Free energy of binding	Inhibition Constant Ki	Electrostatic Energy	Total Interaction molecular Energy	Interaction Surface
Chrysosplenol	-4.97 kcal/mol	229.02 uM	-0.06 kcal/mol	-5.09 kcal/mol	668.594
Linoleic acid	-5.68 kcal/mol	68.12 uM	-0.05 kcal/mol	-8.38 kcal/mol	650.194
Palmitic acid	-4.83 kcal/mol	287.14 uM	-0.06 kcal/mol	-8.23 kcal/mol	654.27
Squalene	-7.28 kcal/mol	4.58 uM	-0.02 kcal/mol	-9.35 kcal/mol	828.053

kcal/mol - kilo calories per mole uM - micro moles

Table 3: Amino acid Residue Interaction of Lead against Tyrosinase (1WX3)

Compound	Interaction	Amino acid residues											
Chrysosplenol	4	38 HIS	42 ILE	54 HIS	182 GLU	184 TRP	188 ASN	190 HIS	191 ASN	194 HIS	195 VAL	206 SER	
Linoleic acid	3	42 ILE	54 HIS	55 ARG	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL			
Palmitic acid	4	38 HIS	42 ILE	54 HIS	184 TRP	190 HIS	191 GLU	194 HIS	195 VAL	206 SER			
Squalene	4	38 HIS	42 ILE	54 HIS	55 ARG	59 PHE	182 GLU	184 TRP	190 HIS	191 ASN	194 HIS	195 VAL	206 SER

HIS- Histidine ILE-Isoleucine GLU-Glutamic acid TRP-Tryptophan ASN-Asparagine VAL-Valine SER-Serine

Compound name	Linoleic acid	Palmitic acid	Squalene	Chrysoplenol
Docking pose				
Plot analysis by 2D interaction				

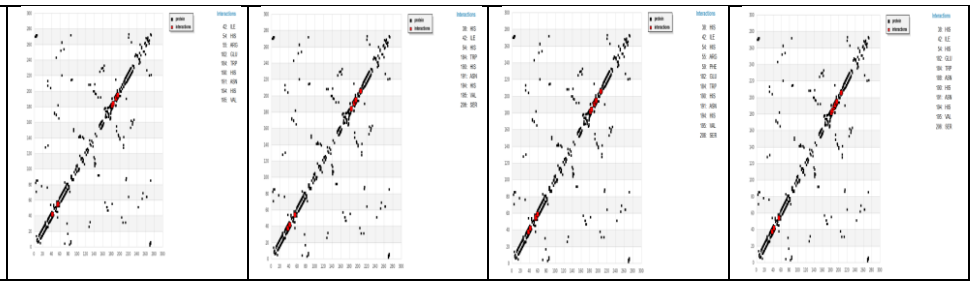
Core amino acid Analysis by Hydrogen bond plotting	

Figure 3: Docking Poses, Plot analysis by 2D interaction and core amino acid analysis by Hydrogen bond plotting of 4 bioactive molecules of the *Sphaeranthus amaranthoides* against the target Tyrosinase (PDB) - 1WX3

Discussion

Vitiligo is an acquired autoimmune disorder characterized by the destruction of epidermal melanocytes, driven by oxidative stress and immune-mediated factors such as TNF-α, Hsp70, and IL-1α, ultimately leading to depigmentation. Excessive production of reactive oxygen species damages melanocytes through protein oxidation, apoptosis, and cytokine activation. Disease activity is often monitored using superoxide dismutase (SOD) levels, which increase during active phases and decline in stable lesions [23] .

Sivakaranthai (*Sphaeranthus amaranthoides*) is classified as a *Karpam* drug and is recognized for its antioxidant properties. Bhogar recommends Sivakaranthai powder for the treatment of dermatitis due to its antihistamine effects [11] .In this context, *Sphaeranthus amaranthoides*, an antioxidant-rich plant, was selected for in silico investigations to explore its therapeutic potential against vitiligo [24] .Molecular docking studies provide valuable insights into ligand–enzyme interactions, identifying the specific amino acid residues involved, the nature of these interactions, and the binding strength as indicated by binding energy values [25].

Computational analysis and literature review identified four major phytocompounds from *Sphaeranthus amaranthoides*—Chrysosplenol, Linoleic acid, Palmitic acid, and Squalene—for docking studies. These ligands demonstrated interactions with key tyrosinase (1WX3) residues, including His38, His54, His63, His190, His194 and His216. Docking scores ranged from –4.83 to –7.28 kcal/mol. Among them, Squalene exhibited the strongest binding affinity (–7.28 kcal/mol), forming four interactions with His38, His54, His190 and His194. Linoleic acid showed the second-highest affinity (–5.68 kcal/mol), interacting with His54, His190 and His194. Chrysosplenol and Palmitic acid displayed binding affinities of –4.97 kcal/mol and –4.83 kcal/mol respectively. Notably, three of the compounds engaged four critical active-site residues, while Linoleic acid interacted with three.

Regarding drug-likeness, with a molecular weight of 360.3 g/mol, a logP of 2.53, three hydrogen bond donors, and eight hydrogen bond acceptors, Chrysosplenol satisfies Lipinski's rule of five [26] .This classification as a lipophilic drug may account for its potential therapeutic effects in conditions such as vitiligo [27]

Conclusion

The computational analysis of *Sphaeranthus amaranthoides* indicates that its bioactive compounds—Chrysosplenol, Palmitic acid, and Squalene—exhibit significant binding affinity to the active site of tyrosinase. These compounds may enhance melanogenesis by synergistically stimulating tyrosinase activity, thereby addressing the melanin deficiency characteristic of vitiligo. Although the findings suggest promising anti-vitiligo potential, further preclinical and clinical studies are required to confirm their mechanisms and therapeutic efficacy. Overall, this study underscores the potential of

Siddha herbal medicine, where centuries of traditional wisdom converge with modern scientific validation to provide safe and holistic approaches for vitiligo management.

Acknowledgement
The authors acknowledge The Tamilnadu Dr.MGR Medical University, Guindy, Chennai

Reference

1. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res. 2012;25(3):E1-13.
2. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol. 2012;51(11):1206-12.
3. Picardo M, Dell'Anna ML, Ezzedine K, Taïeb A, Harris JE, Le Poole IC, et al. Vitiligo. Nat Rev Dis Primers. 2015;1:15011.
4. Shah H, Mehta A, Astik B. Clinical and sociodemographic study of vitiligo. Indian J Dermatol Venereol Leprol. 2008;74(6):701.
5. Taïeb A, Picardo M; VETF Members. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. Pigment Cell Res. 2007;20(1):27-35.
6. Sharma M, Mandal SK. Ayurvedic management of vitiligo (Shvitra). J Ayurveda. 2021;15:249.Available from: https://journals.lww.com/joay/fulltext/2021/15010/ayurvedic_management_of_vitiligo_shvitra.6.aspx
7. Wang J, Pan Y, Wei G, Mao H, Liu R, He Y. Damage-associated molecular patterns in vitiligo: igniter fuse from oxidative stress to melanocyte loss. Redox Rep. 2022;27(1):193-9.
8. Thyagarajan R. L.I.M, Yugi Vaithiya Chinthamani Perunool - 800. 2nd ed. Chennai: Indian Medicine and Homeopathy Department; 1976. p. 356.
9. Chitra SM. Molecular docking study of Siddha herbal formulation Kurudhiazhal Choornam against hypertension. Res J Pharm Technol. 2024;17(9):4228-34. doi:10.52711/0974-360X.2024.00653.
10. CBS BC, SES, JJ, SVS, PN, Joseph Vaiz JM. Evaluation of anti-vitiligo properties through an in-silico computational screening of selected herbal bioactive components with the target protein tyrosinase. Int J Ayurvedic Med. 2024;15(1):154-9. doi:10.47552/ijam.v15i1.4445.
11. Uthamarayan KS. Siddhar Aruvai Maruthuvam. Chennai: Indian Medicine and Homeopathy Department; 1968. p. 122.
12. Bogar. Karpam 300. Chennai: [Publisher unknown]; p. 15.
13. Swarnalathaa Y, Narendran C, Sidhika K. A systemic review of Sphaeranthus amaranthoides and its properties. J Med Plant Herbs. 2023;2:1-5.
14. Thanigavelan V, Venkatachalam L, Kaliyamurthi V, Rajamanickam G, Kumar M. Pharmacological study of a Siddha holistic herb Sivakaranthai - Sphaeranthus amaranthoides Burm for analgesic and anti-inflammatory activities. 2020.
15. Geethalakshmi R, et al. Evaluation of antioxidant and wound healing potentials of Sphaeranthus amaranthoides Burm.f. Biomed Res Int. 2013;2013:607109. doi:10.1155/2013/607109.
16. Gayatri S, et al. Isolation and characterization of chemopreventive agent from Sphaeranthus amaranthoides. Pharmacognosy Res. 2016;8(1):31-5.
17. De S, et al. Phytochemical and GC–MS analysis of bioactive compounds of Sphaeranthus amaranthoides Burm.f. J Pharmacogn Phytochem. 2013;2(1):216-22.
18. Gowri R. GC-MS analysis of phytoconstituents from the ethanol extract of whole plant of Sphaeranthus amaranthoides. Glob J Res Anal. 2024;13(1):1-3.
19. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform. 2009;1:15.

20. Halgren TA. Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94. J Comput Chem. 1996;17(5-6):490-519.
21. Morris GM, Goodsell DS, Halliday RS, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19(14):1639-62.
22. Solis FJ, Wets RJ. Minimization by random search techniques. Math Oper Res. 1981;6(1):19-30.
23. Dhanasekaran S, Selvadoss PP, Manoharan SS. Anti-fungal potential of structurally diverse FDA-approved therapeutics targeting secreted aspartyl proteinase (SAP) of Candida albicans: an in silico drug repurposing approach. Appl Biochem Biotechnol. 2022;195(3):1983-98. doi:10.1007/s12010-022-04207-w.
24. Dhanasekaran S, Selvadoss PP, Manoharan SS, Jeyabalan S, Vijayarangan DR. Revealing anti-fungal potential of plant-derived bioactive therapeutics in targeting secreted aspartyl proteinase (SAP) of Candida albicans: a molecular dynamics approach. J Biomol Struct Dyn. 2023;41(1):1-15. doi:10.1080/07391102.2023.2196703.
25. Vinodini R, Amala Hazel AM, Muthukumar NJ. Siddha management for Venpulli (vitiligo) in pediatrics: a case study. J Pharmacogn Phytochem. 2020;9(1):171-3.
26. Geethalakshmi R, Sakravarthi C, Kritika T, Arul Kirubakaran M, Sarada DV. Evaluation of antioxidant and wound healing potentials of Sphaeranthus amaranthoides Burm.f. Biomed Res Int. 2013;2013:607109. doi:10.1155/2013/607109.
27. Dhrubo J, Sen DJ, Nandi K, Saha D. Rule of five: the five men army to cross the blood brain barrier for therapeutically potent drugs. 2015.