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The activity of vitexicarpin and artemetin as in inhibiting hyperpigmentation: In silico study

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Abstract: Hyperpigmentation, characterized by increased skin darkening, is primarily attributed to augmented melanin production, often exacerbated by UV ray exposure. Inhibiting melanogenesis enzymes, such as tyrosinase, tyrosinase-related protein 1, and d-dopachrome tautomerase, is a recognized strategy for managing hyperpigmentation. Flavonoid compounds, namely vitexicarpin and artemetin, have emerged as potential antihyperpigmentation agents. This study explores the inhibitory capabilities of vitexicarpin and artemetin on melanogenesis enzymes through in silico molecular docking. The process involved optimization of test compounds using HyperChem 8, target protein preparation with Chimera 1.11, method validation, and docking employing AutoDockTools 1.5.6, which integrates Autodock4 and Autogrid4 programs. The validity of the molecular docking method was confirmed with an RMSD value of ≤3 Å. The findings demonstrate that vitexicarpin and artemetin exhibit higher affinity towards tyrosinase, tyrosinase-related protein 1, and d-dopachrome tautomerase than the native ligands. Interaction models between the compounds and target proteins include hydrogen bonds, Van der Waals forces, hydrophobic interactions, and electrostatic bonds, with the most visually identifiable hydrogen bonds. These results suggest that vitexicarpin and artemetin have promising potential as antihyperpigmentation agents by inhibiting melanogenesis enzymes, as evidenced by the molecular docking approach.

Keywords: antihyperpigmentation, artemetin, molecular docking, vitexicarpin

Introduction

Hyperpigmentation, characterized by an increase in melanin production leading to skin darkening, shows a higher prevalence in Asia (21%) compared to other continents [1]. Specifically, the incidence of hyperpigmentation is notably high in Indonesia, attributed to the predominant Fitzpatrick skin types IV and V among Indonesians. These skin types, which rarely burn but tend to tan deeply, along with Indonesia's tropical climate and the region's intense sun exposure, significantly influence the incidence of hyperpigmentation [2].

One of the facial lightening products available is an anti-hyperpigmentation agent, which functions by inhibiting melanin synthesis. This inhibition is achieved through the use of depigmentation agents, specifically tyrosinase inhibitors, which block the activity of the enzyme tyrosinase [3]. The synthesis of melanin involves several key enzymes, including: (i) tyrosinase, which catalyzes the oxidation of the amino acid L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA), and subsequently to dopaquinone; (ii) D-dopachrome tautomerase, which converts dopaquinone to dopachrome and then to 5,6-dihydroxyindole-2-carboxylic acid (DHICA); (iii) Tyrosinase-related protein 1, which transforms DHICA into indole-5,6-quinone carboxylic acid, eventually leading to the formation of eumelanin [4], [5], [6].

Antioxidants are compounds capable of indirectly preventing hyperpigmentation by inhibiting reactive oxygen species (ROS). These compounds neutralize ROS, thereby preventing oxidative damage which is a precursor to melanogenesis. Specifically, in the dermis layer, ultraviolet (UV) light exposure can lead to ROS production, primarily through the lipid peroxidation of melanocyte membranes, subsequently triggering melanogenesis [5]. Among natural substances, legundi leaves (*Vitex trifolia*) are notable for their high antioxidant activity. The ethyl acetate extract of *Vitex trifolia* leaves has been demonstrated to possess an IC_{50} value of 4.2 ppm, indicating potent efficacy in neutralizing ROS [6].

Vitex trifolia leaves are rich in flavonoids, including vitexicarpin and artemetin (Figure 1), which are categorized within the flavonol class. Vitexicarpin,

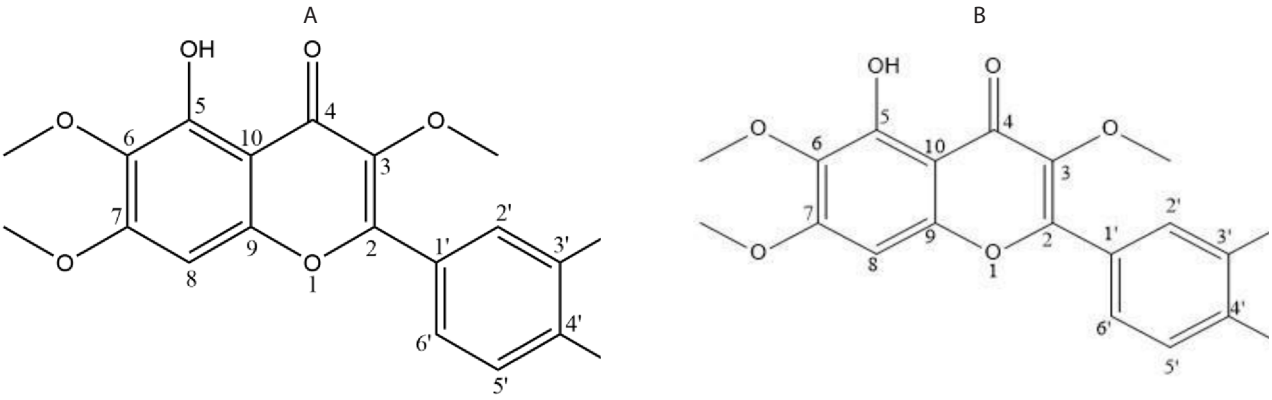

Figure 1. Structure of test compounds. (A) Vitexicarpin, (B) Artemetin

a characteristic marker of *Vitex trifolia* leaves, is present in amounts not less than 0.23% [7]. It has been reported to exhibit diverse biological activities, such as anticancer, antitumor, anti-inflammatory, and immunoregulatory effects [8]. On the other hand, artemetin has demonstrated a significant reduction in hypertension effects by 40-50% in comparison to the control group, which was treated with captopril, a standard hypertension medication [9].

The potential of vitexicarpin and artemetin in inhibiting melanogenesis enzymes—such as tyrosinase, D-dopachrome tautomerase, and tyrosinase-related protein 1—for anti-hyperpigmentation purposes remains unreported. Consequently, preliminary testing using the molecular docking method is essential to explore these compounds as melanogenesis enzyme inhibitors.

Molecular docking, a computational simulation technique, enables the prediction of interactions between proteins (receptors) and compounds (ligands). This method offers several advantages, including the elimination of chemical usage, thereby ensuring safety and reducing research time and costs [10]. It can predict the location, conformation, orientation, and interaction of molecules at the target protein's binding site. The interactions deduced from molecular docking provide critical insights, such as affinity values and interaction models between the ligand and the target protein. Therefore, employing molecular docking is crucial for *in silico* evaluation of vitexicarpin and artemetin as potential skin anti-hyperpigmentation agents.

Methods

The research was conducted at the Laboratory of Pharmaceutical Analysis, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University, employing an *in silico* exploratory research design. This design was specifically chosen to investigate protein targets: tyrosinase, tyrosinase-related protein 1, and D-dopachrome tautomerase, crucial in the study of anti-hyperpigmentation agents. The computational tools utilized included a suite of computers running Windows 7 64-bit, equipped with a range of specialized software for molecular docking and analysis. This software suite comprised the Open Babel GUI program, BIOVIA Discovery Studio Visualizer, and HyperChem8 for molecular modeling and visualization. Additionally, the Chimera 1.11.1 and AutoDock Tools 1.5.6, alongside AutoDock 4 and AutoGrid 4, were employed for detailed docking simulations.

Optimization of the structure of vitexicarpin and artemetin

The structure of vitexicarpin and artemetin was acquisited of their 3-dimensional structures in .sdf format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The first step involved converting these structures into .pdb format using the Open Babel GUI program. Following the conversion, the BIOVIA Discovery Studio Visualizer was employed to verify any structural changes that occurred during the format transition.

Subsequently, the .pdb formatted compounds were subjected to optimization using the HyperChem8 program. This optimization process entailed refining the 3D structures, including all hydrogen atoms, utilizing the Austin Model 1 semi-empirical computational chemistry method. This method encompasses single-point calculations to determine the total energy of the

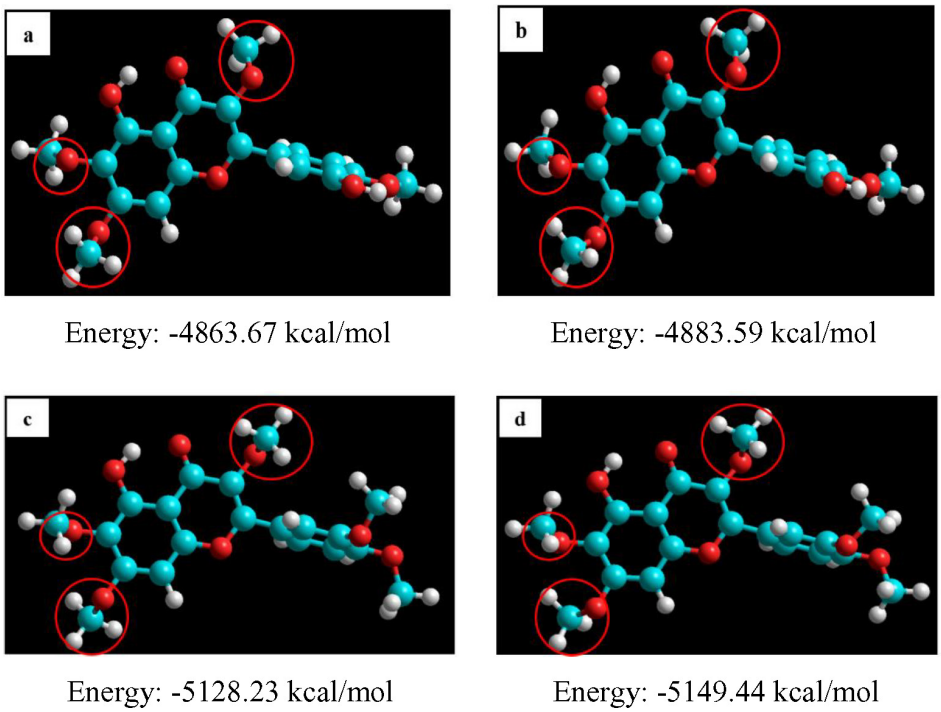

Figure 2. Single-point calculation results and geometry optimization of test compounds
◯ = the change in conformation of the compound after geometry optimization; a = Vitexicarpin single-point conformation; b = Vitexicarpin conformation after geometry optimization; c = single-point artemetin conformation; d = Artemetin conformation after geometry optimization

compound's configuration and geometry optimization to identify the most stable molecular conformation with the lowest total energy. The goal of this optimization is to achieve a molecular structure that accurately reflects the compound's lowest energy state. The optimized structures were then saved in .pdb format for further analysis.

Preparation of protein target

The preparation of protein targets for molecular docking studies commenced with obtaining their active forms that binds to the native ligand. The 3-dimensional structures of tyrosinase (PDB ID: 2Y9X), tyrosinase-related protein 1 (PDB ID: 5M8M), and D-dopachrome tautomerase (PDB ID: 3KAN) were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The initial preparation step involved the removal of water molecules from the structure. Following this, the native ligand associated with each target protein was separated using the Chimera 1.11.1 program. The removal of the native ligand serves a crucial purpose: it creates a vacant pocket cavity, which is necessary for the forthcoming docking simulations. This step not only makes space available for the test compounds but also allows for the identification of the pocket shape, pocket coordinates, binding site center, and cavity radius.

Validation of the molecular docking method

The validation of the molecular docking protocol, crucial for ensuring the accuracy and reliability of the simulation results, was performed using the AutoDock program. This method validation involved a critical step known as redocking, where the native ligand of each target protein—previously removed during protein preparation—was docked back into its corresponding protein target. A key parameter in this validation process is the Root Mean Square Deviation (RMSD), with a threshold value set at ≤ 3.0 Å.

Docking of vitexicarpin and artemetin

The test compounds, in optimized .pdb format, were docked onto each prepared target protein using the AutoDock application. The docking results are expressed as bond energy values and types of hydrogen bonds formed between the test compound and the

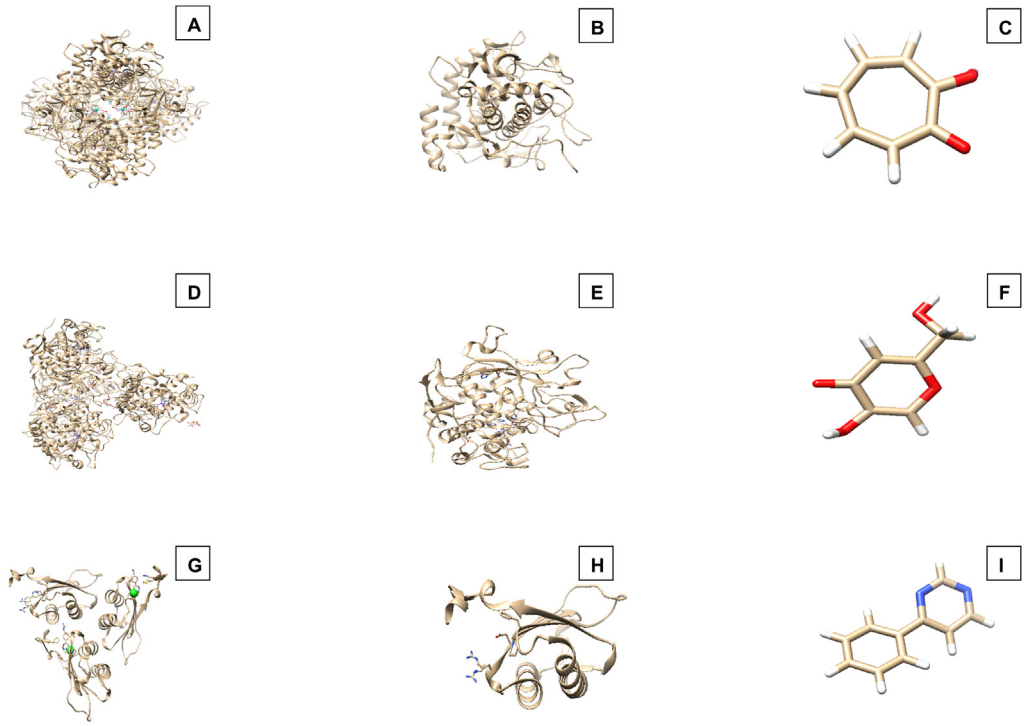

Figure 3. Target protein chain structure and native ligand. (A) Protein tyrosinase, (B) tyrosinase B chain without native ligand, (C) Native ligand 0TR, (D) Tyrosinase related protein 1, (E) Tyrosinase-related protein 1 chain A, (F) native ligand KOJ, (G) Protein D-dopachrome tautomerase, (H) C D-dopachrome tautomerase chain, (I) Native ligand RW1; the color of the native ligand, red = O atom; blue = N atom

target protein, which are then analyzed through a visualization process.

Results

Optimization compounds structure

Based on the superposition results, there is no structural change in the test compound when converting formats, allowing the continuation with optimization (Figure 2). The geometry optimization of the test compounds reveals a lower total energy value compared to the single-point calculation results, indicating that the optimization process has been successfully executed.

Preparation of protein target

The protein targets comprise three enzymes involved in melanogenesis: tyrosinase (PDB ID: 2Y9X), tyrosinase-related protein 1 (PDB ID: 5M8M), and D-dopachrome tautomerase (PDB ID: 3KAN). The structures of the proteins, a chain without the native ligand, and the structure of each protein's native ligand, are presented in Figure 3.

Method validation

Setting the grid box was conducted to define the search space within the coordinate system on the target protein, where the interaction occurs. The values for the grid size and grid center on the target protein grid box are detailed in Table 1. The validation method yielded the 10 best conformations, alongside their RMSD values and bond energies. Root Mean Square Deviation (RMSD) is commonly used to compare the conformational differences in molecular systems, including in molecular dynamics and protein-ligand docking. The selected conformation is the one with the lowest RMSD value that also satisfies the validation criteria (RMSD $\leq$ 3 Å).

Docking of test compounds on protein target

The docking results of the test compound with the target protein yielded bond energy, as well as the presence of hydrogen bonds formed from the interaction of the test compound with the protein (Table 1, Table 2). The visualization of these interactions between the test compounds and each target protein is displayed in Figure 4.

Table 1. Grid box arrangements on protein target

Protein target	Grid box		RMSD (Å)
	Grid size	Grid center	
Tyrosinase (2Y9X)	x = 50 y = 50 z = 54	x = 2.798 y = 7.111 z = -8.073	0.99
Tyrosinase related protein 1 (5M8M)	x = 70 y = 50 z = 60	x = -11.972 y = 3.361 z = -7.806	2.25
D-dophachrome tautomerase (3KAN)	x = 60 y = 60 z = 60	x = 2.472 y = -6.222 z = 1.361	2.42

RMSD: Root Mean Square Deviation

Table 2. Docking results between protein target and test compounds

Protein target	Ligand	Bond energy (Kcal/mol)	Amino acid residue	Groups in hydrogen bonds (Protein-Ligand)
Tyrosinase (2Y9X)	Native ligand	-4.92	His61	HE2-OA2
	Vitexicarpin	-5.28	Glu16	HN-O
	Artemetin	-5.44	Gln114	HE21-O
Tyrosinase-related protein 1 (5M8M)	Native ligand	-5.15	His192 His224	HE2-O6 HE2-O6
	Vitexicarpin	-5.78	Val89	HN-O
	Artemetin	-5.35	His75	HN-O
D-dopachrome tautomerase (3KAN)	Native ligand	-6.46	Ile64	HN-N3
	Vitexicarpin	-6.48	Asn73	HD21-O
	Artemetin	-6.49	Asn73	HN-O

His = histidine; Glu = glutamic acid; Gln = glutamine; Lys = lysine; Arg = arginine; Val = Valin; Ile = isoleucine; Asn = asparagine

HE2-OA2 = hydrogen atom in the E2 position (epsilon number 2) on the amino acid residue binds to the oxygen atom in the A2 position on the native ligand

HN-O = hydrogen atom in the N position (nitrogen branch) on the amino acid residue binds to the oxygen atom in the native ligand/test compound

HE21-O = hydrogen atom position E21 (epsilon number 21) on the amino acid residue binds to the oxygen atom in the test compound

HZ2-O = hydrogen atom position Z2 (zeta number 2) on the amino acid residue binds to the oxygen atom in the test compound

Table 3. Bond energy quantification results

Protein target	Ligand	Energy Vdw_ Hb_ Desolv (kcal/mol)	Energy elec (kcal/mol)	Final total internal energy (kcal/ mol)	Torsional free energy (kcal/ mol)	Unbound system's energy (kcal/ mol)
Tyrosinase (2Y9X)	Native ligand	-4.85	-0.07	+0.00	+0.00	+0.00
	Vitexicarpin	-5.22	-0.06	-0.52	+0.00	-0.52
	Artemetin	-6.55	-0.08	-0.71	+1.19	-0.71
Tyrosinase-related protein 1 (5M8M)	Native ligand	-5.56	-0.09	-0.08	+0.50	-0.08
	Vitexicarpin	-6.31	-0.37	-0.59	+0.90	-0.59
	Artemetin	-6.21	-0.30	-0.73	+1.16	-0.73
D-dophachrome tautomerase (3KAN)	Native ligand	-6.66	-0.10	-0.23	+0.30	-0.23
	Vitexicarpin	-7.42	-0.05	-0.47	+0.99	-0.47
	Artemetin	-7.05	-0.12	-0.71	+0.68	-0.71

Vdw: Van der Waals bonding; Hb: hydrogen bonding; Desolv: hydrophobic bonding; Elec: Electrostatic bonding

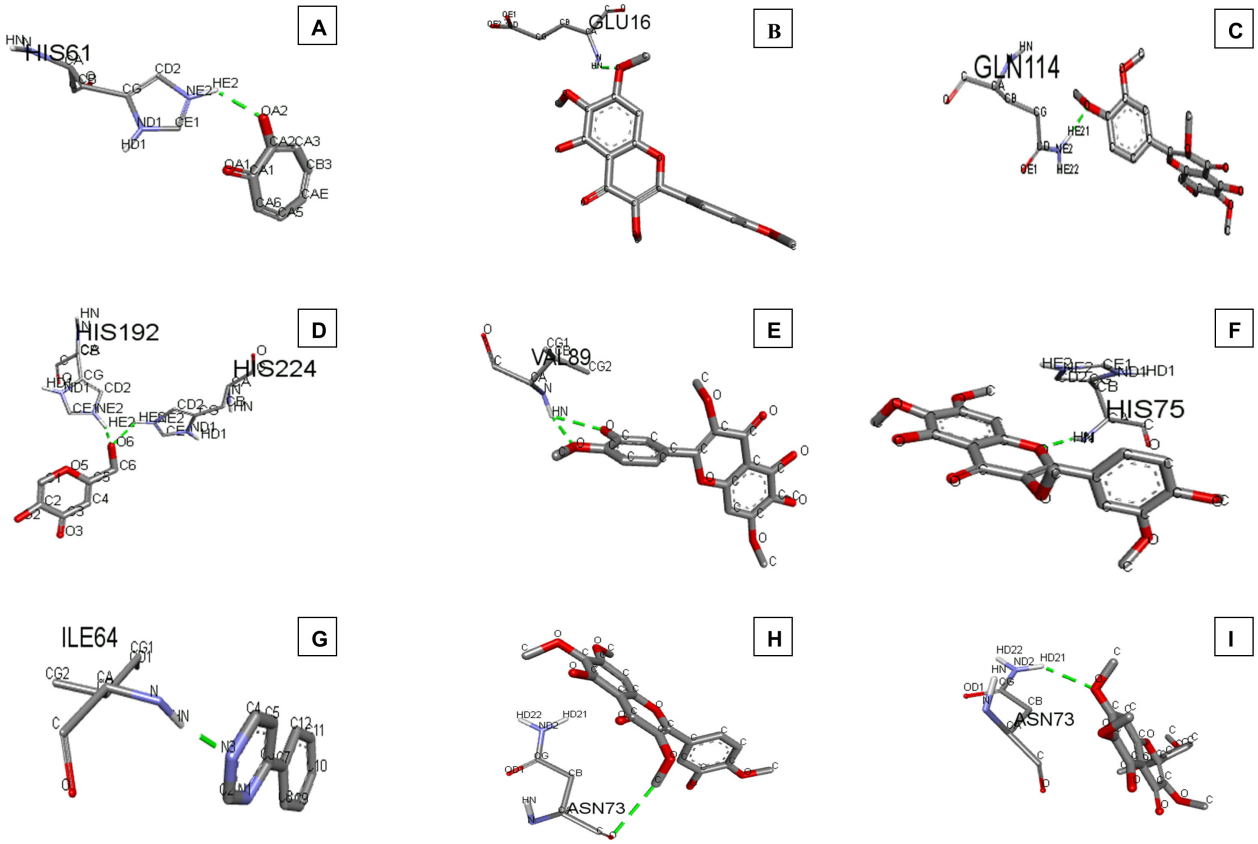

Figure 4. Visualization of native ligand and test compounds on protein targets. (A) Hydrogen bonding between the tyrosinase and the 0TR native ligand, (B) vitexicarpin, (C) artemetin; (D) Hydrogen bonding between the target protein tyrosinase related protein 1 and the native ligand KOJ, (E) vitexicarpin, (F) artemetin; (G) hydrogen bonding between d-dopachrome tautomerase and native ligand RW1, (H) vitexicarpin, (I) artemetin

Discussion

The docking results demonstrate that both vitexicarpin and artemetin exhibit bond energies lower than those of native ligands for all three enzymes, suggesting a stronger affinity towards the protein targets.

The geometry optimization of the compounds reveals lower total energy values compared to single-point calculation results. A lower energy level in a compound enhances its ability to donate electrons, thereby facilitating easier binding to the protein target. The selection of the target protein chain was guided by the binding site of the native ligand, which exhibits inhibitory activity towards the target protein. For tyrosinase, chain B was chosen, featuring the native ligand tropolone (0TR), as all four chains of tyrosinase share this same native ligand. For tyrosinase-related protein 1, chain A was selected, with native ligand kojic acid (KOJ), and for D-dopachrome tautomerase, chain C was selected, with native ligand RW1.

The docking results indicated the formation of hydrogen bonds resulting from the interaction between the test compound and the protein. A negative bond energy between the test compound and the target protein suggests an affinity towards the target protein, with more negative bond energy values indicating a stronger and more stable bond (affinity) between the test compound and the enzyme.

The variance in amino acid residues involved in the hydrogen bonding interaction between the native ligand and the test compound with the target protein is attributed to differences in the 3D conformational structures of the native ligand and the test compound. These differences determine the most stable state of binding to the target protein's binding site. Besides hydrogen bonds, the bond energy value is also affected by Van der Waals interactions, hydrophobic interactions, electrostatic forces, total internal energy, torsional free energy, and the system's unbound energy. Van der Waals bonds contribute significantly to the overall bond between the target protein and the compound through atomic interactions. Electrostatic interactions, effective over longer distances than other interaction types, are durable and contribute a ΔG value of -5 kcal/mol. These interactions involve charged amino acid residues such as aspartic acid (Asp) and glutamate (Glu), which are negatively charged, and arginine (Arg), lysine (Lys), and histidine (His), which are positively charged [11].

Hydrophobic interactions occur between two non-polar groups in a compound and a non-polar group in the receptor, each surrounded by water molecules. These interactions, along with Van der Waals and electrostatic forces, play a crucial role in stabilizing the bond between the target protein and the compound.

Conclusion

Vitexicarpin and artemetin exhibit an affinity for the target proteins involved in melanogenesis—namely, tyrosinase, tyrosinase-related protein 1, and D-dopachrome tautomerase—as evidenced by more negative bond energy results. These findings suggest that vitexicarpin and artemetin have potential as anti-hyperpigmentation agents through their mechanism of inhibiting melanogenesis enzymes.

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Declaration of interest

The authors declare no conflict of interest

Author Contribution

IKRR and NPLL conceptualized the study design, NLAKA and NMPS investigated the data, IKRR and NLAKA wrote original draft, IKRR, NLAKA, NMPS, NPLL reviewed and edited the final version, NPLL supervised all experiment. All authors have read the final manuscript.

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