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Comparative *in-silico* analysis of vitexin and orientin as potential antiphotoaging agents against MMP enzymes

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Abstract: Photoaging, a result of excessive UV exposure, increases ROS production and collagen degradation by MMPs, causing skin wrinkles and roughness. This study explores the potential of vitexin and orientin as natural antiphotoaging agents through in-silico molecular docking, comparing their efficacy against retinol in inhibiting MMP-1, MMP-3, and MMP-9 enzymes involved in photoaging. The research utilized Hyperchem 8 for compound optimization, Chimera 1.11 for target protein preparation, and AutodockTools 1.5.6 for docking analysis. Results demonstrated that vitexin and orientin exhibit stronger affinity towards MMP-1, MMP-3, and MMP-9, indicated by more negative binding energies than retinol. Their interaction with the MMP enzymes, characterized by specific hydrogen bonds with key amino acid residues, suggests a potent inhibitory effect. This affinity indicates vitexin and orientin's potential as effective antiphotoaging agents, providing a basis for further exploration in skin care applications.

Keywords: antiphotoaging, matrix metalloproteinase, molecular docking, orientin, vitexin

Introduction

Extrinsic skin aging, prominently influenced by UV radiation, is commonly called photoaging [1]. This condition manifests through symptoms such as wrinkled skin, uneven pigmentation, and skin roughness [2]. Notably, the prevalence of photoaging has shown a significant increase over time. A continuous 15-year study in Nambour highlighted a substantial rise in severe photoaging cases, from 42% of the population in 1992 to 88% in 2007 [3]. Such data underscores the escalating impact of photoaging on public health.

The molecular mechanisms underlying photoaging involve prolonged exposure to UVB and UVA rays, leading to an increased production of intracellular reactive oxygen species (ROS). These ROS activate the mitogen-activated protein kinase (MAPK) pathway, culminating in activator protein-1 (AP-1) formation. AP-1 subsequently regulates the transcription of matrix metalloproteinases (MMPs) — specifically MMP-1, MMP-3, and MMP-9 — which degrade collagen types I, III, and IV. This degradation process primarily contributes to the wrinkling and roughness characteristic of photoaged skin [5].

Retinol has emerged as a widely adopted compound in cosmetics for its efficacy in countering photoaging. By decelerating the aging process, retinol enhances skin texture and reduces dryness and fine lines through collagen protection. It notably inhibits the activity of MMP-1, MMP-3, and MMP-9, thus safeguarding the skin's structural integrity [6][7].

Antioxidants indirectly mitigate MMP expression by reducing ROS production [8]. In this context, tamarind leaves (*Tamarindus indica*), rich in antioxidants, present a promising avenue for photoaging prevention. These leaves comprise polyphenolic compounds [9], with vitexin and orientin being the predominant flavonoid-C-glycoside compounds identified [10]. Notably, vitexin and orientin constitute the most significant polyphenolic content in tamarind leaves, measured at 8.99% and 4.8 mg/g dry extract, respectively [11][12]. Both compounds exhibit significant antioxidant activities, suggesting their potential in anti-photoaging applications.

Vitexin, a biomarker compound in tamarind leaves, has shown promise in inhibiting UVA-induced aging in human dermal fibroblasts by targeting specific residues in the MAPK1, thus mitigating UVA-irradiated skin photoaging [13]. Similarly, orientin further showcases

its potent antioxidant activity through the DPPH assay, achieving an IC_{50} value of 0.84 μM. This performance is notably superior to that of recognized antioxidant standards such as trolox (IC_{50} = 0.97 μM) and ascorbic acid (IC_{50} = 0.93 μM) [14].

Molecular docking, a pivotal method in structure-based drug design (SBDD), enables the study of intermolecular interactions through computational techniques [15]. This method is essential for predicting the binding mode and affinity of ligands to target proteins, facilitating the efficient discovery of potential therapeutic agents [16].

Given the antioxidant capabilities of vitexin and orientin, their roles in inhibiting MMP-1, MMP-3, and MMP-9 require further investigation. The *in-silico* approach, employing molecular docking, offers a preliminary yet insightful analysis of the interactions between these compounds and target proteins [17]. This methodological choice promises a cost-effective, time-efficient pathway [18] to uncovering the anti-photoaging potential of vitexin and orientin.

Methods

Preparation and optimization of 3D structure test compounds and positive control

The three-dimensional (3D) structures of the test compound and the positive control were initially sourced from the National Center for Biotechnology Information's PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format. These structures underwent conversion to .pdb format utilizing the Open Babel GUI program. Subsequent optimization of the 3D structures, including their hydrogen atoms, was conducted using the HyperChem 8 software. This optimization employed the AM1 semi-empirical computational method, incorporating single-point calculations and geometry optimization, to refine the molecular structures. The optimized compounds were then saved in .pdb format for further analysis.

Target protein preparation

The target proteins for the docking studies, MMP-1 (PDB ID: 966C), MMP-3 (PDB ID: 1G4K), and MMP-9 (PDB ID: 2OW0), were downloaded from the Protein Data Bank at http://www.rcsb.org/pdb/home/home.do. These proteins were selected based on their active form structures that bind to native ligands. The target proteins' preparation commenced with removing water molecules from their structures using the Chimera 1.11 software. Each enzyme chain was then isolated from its native ligand to delineate the binding site, allowing for the determination of pocket coordinates, binding site center, and cavity radius. This preparation was essential for the subsequent validation and docking of the test compounds, with chain selection guided by existing data on the binding efficacy of native ligands.

Molecular docking validation

The molecular docking process was validated using the AutodockTools 1.5.6 suite, which includes the Autodock4 and Autogrid4 programs. Before validation, the grid size and center settings were established using Autogrid4. The validation for each MMP-1, MMP-3, and MMP-9 involved redocking the native ligand into the target protein from which it was removed, employing grid dimensions of x = 40, y = 40, and z = 40.

Docking of test compounds and positive control on target proteins

Following their optimization, test compounds and the positive control were docked onto the prepared target proteins using the AutodockTools 1.5.6 suite, again leveraging Autodock4 and Autogrid4 programs. This docking utilized the same grid box settings established during the validation process to ensure accuracy and consistency in assessing compound efficacy and interaction.

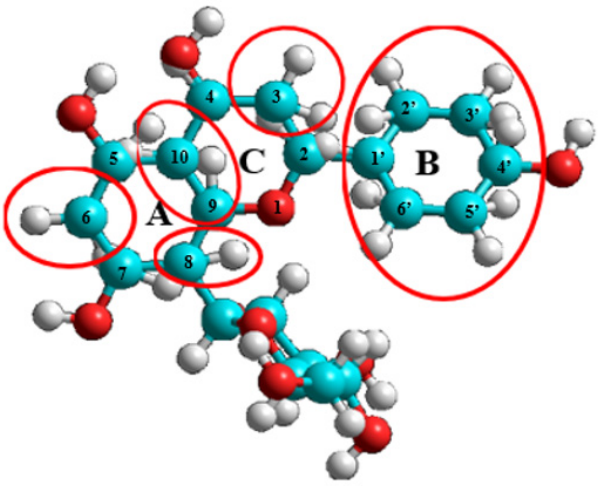
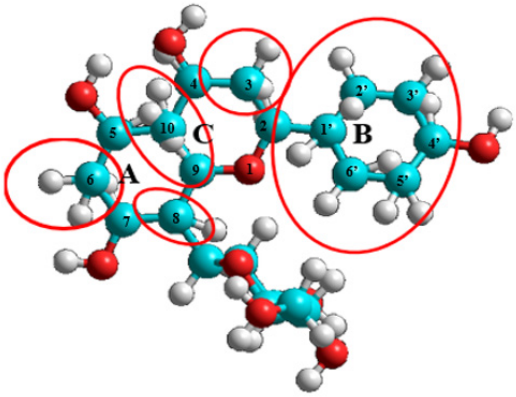
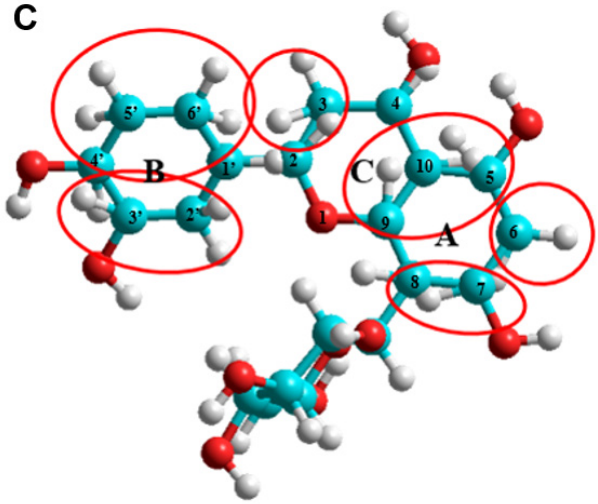
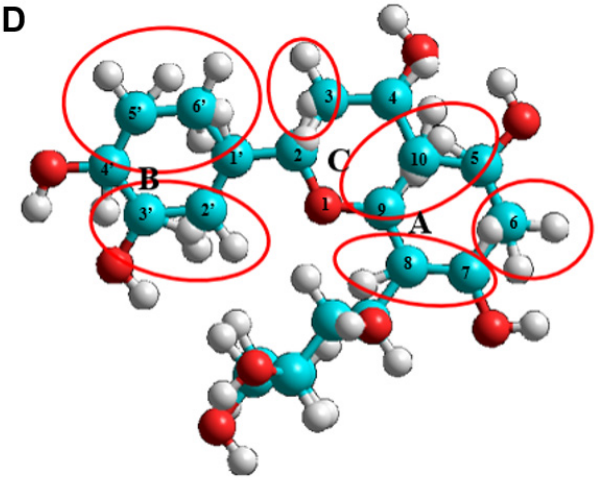
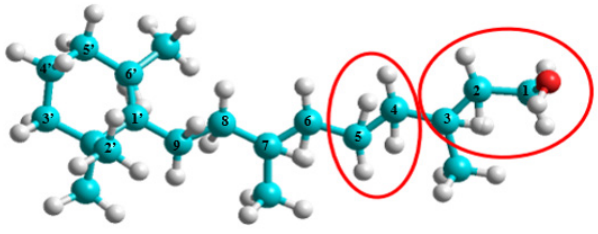
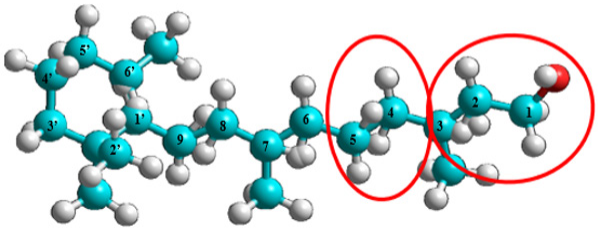
Results

Preparation and optimization of 3D structure test compounds and positive control

The initial step in the computational analysis involved converting the 3D structures of vitexin, orientin, and retinol from .sdf to .pdb format. This process was facilitated using the Open Babel GUI program. To ensure the fidelity of the conversion, the BIOVIA Discovery Studio Visualiser was employed to compare the .sdf and .pdb structures. Observations confirmed that the transition to .pdb format did not induce any structural alterations, such as broken bonds or changes in bond lengths, thus maintaining the integrity of the compounds for subsequent optimization.

Following this verification, the Hyperchem 8 program was utilized to optimize the test compounds using the AM1 semi-empirical method. This optimization process

Table 1. Single-point calculation results and geometry optimization

Single point	Geometry optimization
A 	B 
Energy= -5579.291043 kcal/mol	Energy= -5592.9947 kcal/mol
C 	D 
Energy= -5681.688920 kcal/mol	Energy= -5696.2372 kcal/mol
E 	F 
Energy= -5057.943785 kcal/mol	Energy= -5072.2823 kcal/mol

= Changes in compound conformation after geometric optimization. (A) Initial vitexin conformation, (B) Vitexin conformation optimization, (C) Initial orientin conformation, (D) Orientin conformation optimization, (E) Initial retinol conformation, (F) Retinol conformation optimization

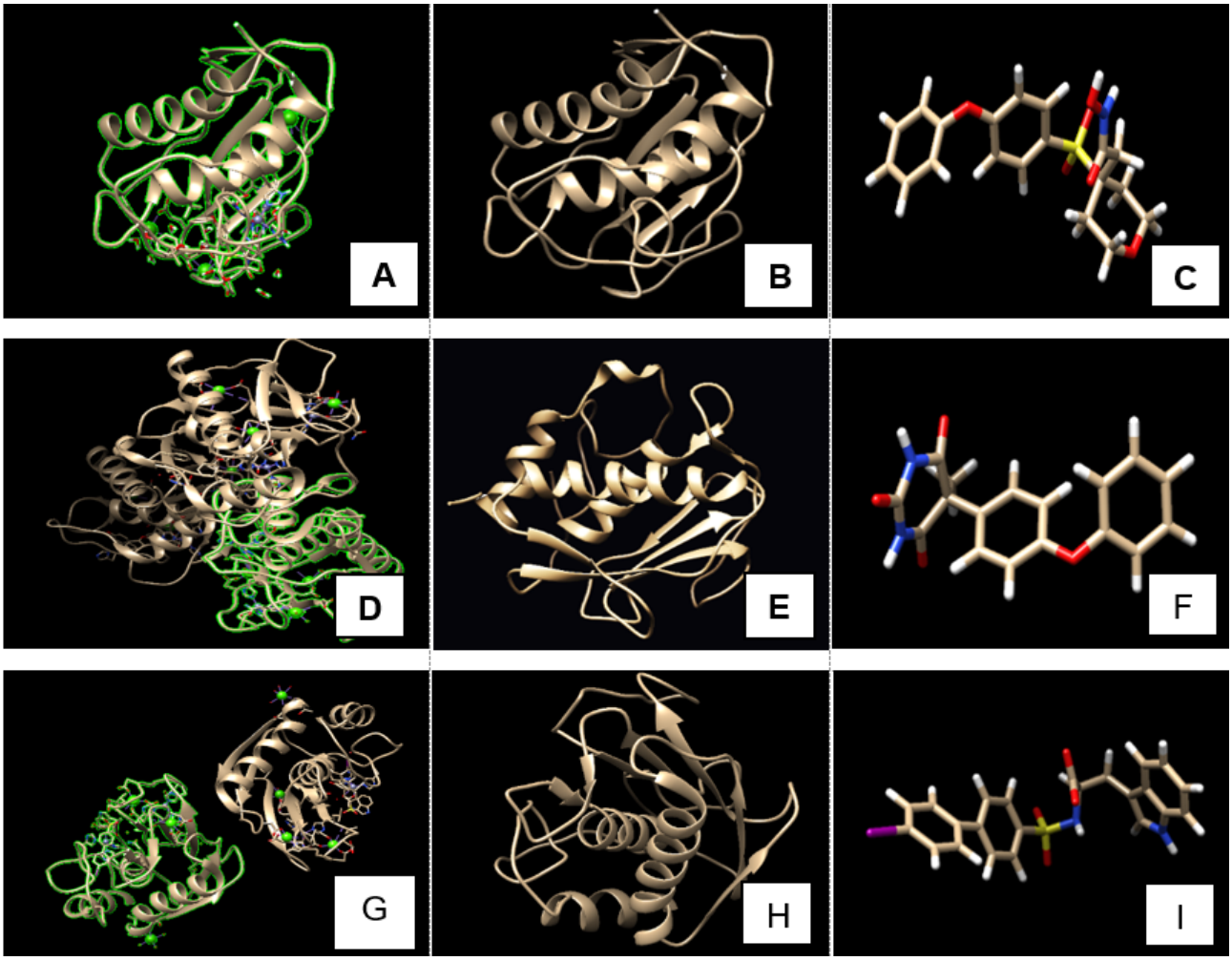


Figure 1. Structure of target protein chains and their native ligands visualized with Chimera 1.11. (A) MMP-1 structure, (B) A Chain MMP-1, (C) Native ligand RS2, (D) MMP-3 structure, (E) A Chain MMP-3, (F) Native ligand HQQ, (G) MMP-9 structure, (H) A Chain MMP-9, (I) Native ligand 6MR, = carbon atom, = sulfur atom, = oxygen atom, = nitrogen atom, = iodine atom, = hydrogen atom

comprised single-point calculations and geometry optimization, aiming to refine the compounds' structures for accurate docking simulations. Notable modifications were observed in the geometric configurations of the hydrogen atoms across the molecular structures of vitexin, orientin, and retinol (Table 1):

- Vitexin exhibited alterations in the hydrogen atoms positioned on the 3rd, 6th, 8th, 9th, and 10th carbons of the A and C rings, as well as on the carbon atoms of the B ring.
- Orientin showed changes in the hydrogen atoms of the 3rd, 5th, 6th, 7th, 8th, 9th, and 10th carbons on the A and C rings and the 2nd, 3rd, 5th, and 6th carbon atoms on the B ring.
- Retinol underwent geometric modifications in the hydrogen atoms of the 1st, 2nd, 3rd, 4th, and 5th carbons and the OH atom on the 1st carbon.

Target protein preparation

The preparation of target proteins MMP-1 (PDB ID: 966C), MMP-3 (PDB ID: 1G4K), and MMP-9 (PDB ID: 2OW0) was conducted to ensure their readiness for molecular docking studies. This preparation involved two critical steps: removing water molecules from each protein's selected chain and separating each protein from its native ligand. This process was essential to define the active sites for docking, allowing for interactions between the test compounds and the target proteins (Figure 1).

Molecular docking method validation

Validation was achieved through redocking native ligands into MMP-1, MMP-3, and MMP-9, resulting in ten conformations per protein. Conformations with the lowest root mean square deviation (RMSD)

Table 2. Results of redocking native ligand on MMP-1, MMP-3, and MMP-9

Target proteins	Conformation	RMSD (Å)
MMP-1	6	0.99
MMP-3	9	1.08
MMP-9	9	1.22

Table 3. Docking results between target protein and native ligand, test compounds, and positive control

Target proteins	Ligand	Bond free energy (kcal/mol)	Hydrogen bonds	Groups in hydrogen bonds (protein-ligand)
MMP-1	Native ligand	-10.31	Asn73 Leu74 Ala75 His111 Glu112 His121	HD22-O25 HN-O26 HN-O25 HE2-O31 OE2-HOX HE2-O31
	Vitexin	-10.40	Leu74 Ala75 Thr134	HN-O HN-O O-H15
	Orientin	-10.37	Asn73 Leu74 Ala75 Thr134	HD22-O HN-O HN-O O-H15
	Retinol	-9.72	Asn73	HD22-O
MMP-3	Native ligand	-10.35	Leu82 Ala83 His119 His123 His129	HN-O6 HN-O6 HE2-O2 HE2-O2 HE2-O2
	Vitexin	-10.39	Leu82 Ala83	HN-O HN-O
	Orientin	-10.57	Leu82 Ala83 Leu136 Tyr141	HN-O O-H2 O-H10 O-H17
	Retinol	-10.24	His142	HE2-O
MMP-9	Native ligand	-11.62	Leu79 Ala80 Gln118	HN-OAP HN-OAP; O-HN HE21-OXT
	Vitexin	-10.35	Leu79 Ala80 Gln118	HN-O HN-O OE1-H19
	Orientin	-10.54	Leu79 Ala80 Gln118 Tyr139	HN-O HN-O HE22-O HN-O
	Retinol	-10.34	Leu79	HN-O

values—meeting the ≤ 3Å criterion—were selected for subsequent analyses: 0.99Å (MMP-1, conformation 6), 1.08Å (MMP-3, conformation 9), and 1.22Å (MMP-9, conformation 9) (Table 2).

Docking of test compounds and positive control on target proteins

Docking experiments, validated by RMSD parameters (≤ 3Å), yielded ten conformations for each compound-

protein interaction. Selection criteria for the optimal conformation included the lowest bond-free energy (ΔG), indicative of a strong and stable bond. For vitexin, the optimal conformations were identified as conformation 8 (MMP-1 and MMP-3) and conformation 2 (MMP-9). Orientin showed best fit with conformation 1 (MMP-1), conformation 4 (MMP-3), and conformation 5 (MMP-9). Retinol's docking yielded conformation 5 (MMP-1), conformation 7 (MMP-3), and conformation 3 (MMP-9) as the most favorable. Table 3 encapsulates the docking results, including bond-free energy values and visualizations of hydrogen bonding between test compounds and target protein amino acid residues.

Discussion

This study is an *in-silico* investigation into vitexin and orientin, highlighting their promising roles as anti-photoaging agents through inhibiting MMP-1, MMP-3, and MMP-9 enzymes. The evaluation was based on the analysis of bond-free energy and interaction models, which compared these compounds with retinol, an established anti-aging agent known for its MMP inhibitory mechanism.

The results revealed that both vitexin and orientin exhibit strong affinities for MMP-1, MMP-3, and MMP-9, as indicated by their negative binding free energies. Notably, these compounds demonstrated a more negative bond-free energy than retinol and the native ligands for MMP-1 and MMP-3, suggesting a superior binding efficiency and potential inhibitory effect. Although their affinity for MMP-9 was slightly weaker than that of the native ligands, the proximity of their bond-free energy values to those of the native ligands suggests a considerable potential for inhibition.

Comparative analysis with retinol showed vitexin and orientin have more negative binding free energies for all three MMP enzymes, indicating their superior anti-photoaging potential. This is further supported by the specificity of their interactions at the enzyme active sites, primarily through hydrogen bonding with key amino acid residues essential for enzymatic activity [19]. The binding of vitexin and orientin to the same active sites as the native ligands, involving critical residues such as asparagine, leucine, alanine, histidine, and glutamic acid, demonstrates their capability to inhibit the MMP enzymes effectively.

Hydrogen bonding patterns further illustrate the specificity of the interactions between vitexin, orientin, and the MMPs, contrasting with the more limited interaction observed for retinol. This specificity highlights the potential of vitexin and orientin as effective MMP inhibitors and suggests their advantages over retinol in targeting the enzymatic processes underlying photoaging.

Quercetin, another polyphenolic compound with known anti-inflammatory properties, demonstrates similar inhibitory activity against MMPs [20]. The comparative analysis with quercetin underscores the broader potential of polyphenolic compounds in anti-photoaging and anti-inflammatory applications. The docking results indicate that polyphenolic compounds, including vitexin and orientin, exhibit negative binding energy values with MMP enzymes, highlighting their potential to inhibit these enzymes effectively.

Conclusion

The *in-silico* findings suggest that vitexin and orientin have significant potential as anti-photoaging agents, with stronger affinities for MMP-1, MMP-3, and MMP-9 than the established anti-aging agent retinol. The detailed interaction models reveal specific amino acid residues critical for inhibiting these enzymes, further validating the potential of vitexin and orientin as effective MMP inhibitors. While these results are promising, further *in vivo* and clinical trials are necessary to explore the anti-photoaging effects of vitexin and orientin fully. Such studies will provide a more comprehensive understanding of their efficacy and mechanisms of action in preventing skin aging.

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Declaration of interest

None.

Author contributions

RPBN, NPLL conceptualized the study design; NMRW, NMPS investigated the data, RPBN, NMRW wrote the original draft. All authors that were mentioned reviewed, edited and read the final version of this manuscript.

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