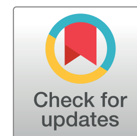
Pharmacy Reports https://doi.org/10.51511/pr.58

RESEARCH ARTICLE Open Access

# The potency of pinostrobin and pinocembrin as antiphotoaging agents: *in silico* study



**I Gusti Ngurah Agung Pradnyana, Ketut Yuantarisa Kartika Putri, Ni Made Pitri Susanti, Ni Putu Linda Laksmiani***

Department of Pharmacy, Faculty of Mathematics and Natural Science, Udayana University, Bukit Jimbaran, Badung, Bali 80361, Indonesia

*Corresponding author: Email: laksmini@unud.ac.id

**Abstract:** Photoaging occurs when the skin ages due to ultraviolet light exposure. Phenolic compounds generally possess antioxidant activity, which helps prevent the formation of free radicals caused by sunlight exposure. This study explores the potential of pinostrobin and pinocembrin as antiphotoaging agents through molecular docking against matrix metalloproteinases (MMPs): MMP-1, MMP-3, and MMP-9. We utilized Hyperchem 8 to prepare and optimize the test compound and Chimera 1.11.1 for protein preparation. Validation and docking procedures were conducted using the AutoDockTools 1.5.6 application, with validation confirming that the method was valid with an RMSD value ≤ 3 Å. Both pinostrobin and pinocembrin exhibited an affinity for the target protein, although their affinity was slightly less than that of the native ligand and retinol. In conclusion, pinostrobin and pinocembrin demonstrate an affinity for MMP-1, MMP-3, and MMP-9, indicating their potential as anti-photoaging agents by obstructing the mechanisms of MMP-1, MMP-3, and MMP-9.

**Keywords**: antiphotoaging, matrix metalloproteinase, pinocembrin, pinostrobin

## Introduction

Photoaging refers to the skin aging caused by ultraviolet (UV) light exposure. Photoaging is responsible for approximately 80% of cases of skin aging [1]. UVB and UVA radiation induce oxidative stress by generating of reactive oxygen species (ROS). These ROS can activate cytokine receptors and TGF-β receptors, subsequently leading to an increase in the production of activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB), thereby increasing the production of matrix metalloproteinase (MMP) [2]. This MMP plays a disruptive role in regulating the skin's cellular matrix, causing collagen degradation. A decrease in the collagen amount within the skin's dermis causes wrinkles and premature skin aging, which manifests as epidermal hyperplasia.

Three types of MMP, namely MMP-1 (interstitial collagenase or collagenase 1), MMP-3 (stromelysin 1), and MMP-9 (gelatinase B), experience an increase in production within the skin due to UV exposure. MMP-1 initiates the degradation of collagen types I and III, while MMP-3 degrades the basement membrane of type IV collagen and activates proMMP1. Consequently, collagen broken down by MMP-1 undergoes further degradation by MMP-3. Meanwhile, MMP-9 contributes to the breakdown of collagen fragments generated by collagenase [3]. Continuous exposure to sunlight causes humans susceptible to photoaging. Thus, an anti-photoaging agent becomes necessary to maintain and protect the skin health, preventing premature aging.

Antioxidants are compounds capable of reducing oxidation reactions triggered by various oxidative agents, such as ROS, formed due to sun exposure. They can effectively prevent damage caused by free radicals. Plant-based sources often contain antioxidant activity in the form of phenolic compounds. Two such compounds, pinostrobin and pinocembrin, are present in *Rotunda boesenbergia* and *Boesenbergia pandurata* Roxb. Pinocembrin was reported for its antioxidant activity. This is evident from a comparison with the $H_2O_2$-induced group, demonstrating the impact of antioxidant activity within a range of 5 to 40 μM. In contrast, the compound exhibits a pro-oxidant effect at higher concentrations, ranging from 5 μM (294.56 ± 7.6%) to 40 μM (179 .94 ± 6.2%). In contrast, it showed a pro-oxidant effect in a high concentration range, from 60 μM to 80 μM [4]. Given the similar structure of pinocembrin and pinostrobin (Figure 1), the antioxidant activity displayed by pinocembrin

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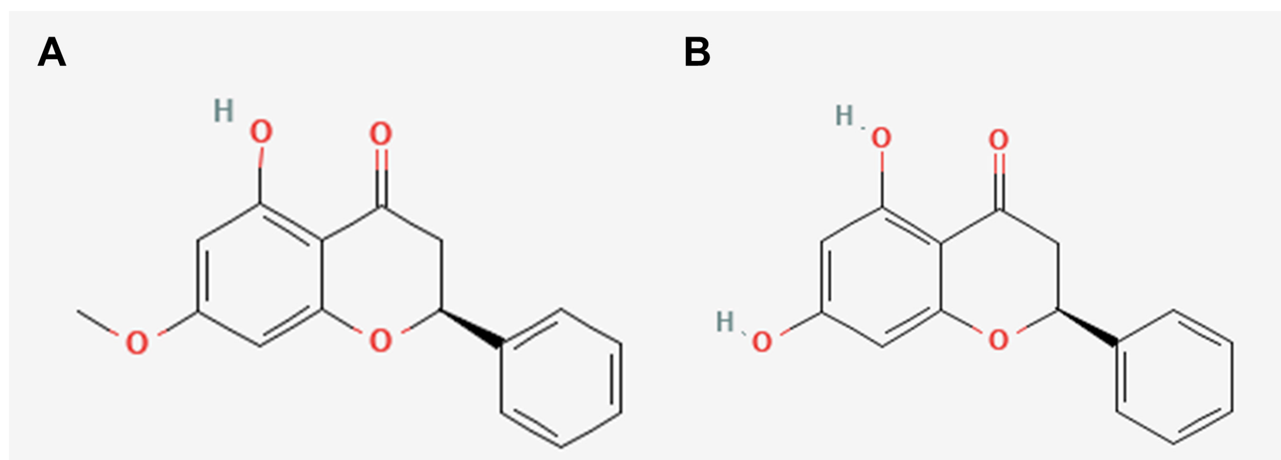


**Figure 1.** Structure of pinostrobin and pinocembrine

underscores the potential of these two compounds as agents against photoaging.

The molecular docking method can predict the position, conformation, orientation, and interaction of a molecule within the binding site of a protein target. This process yields affinity values and models depicting how the ligands interact with the protein target [5]. Utilizing this affinity and interaction model, it becomes feasible to demonstrate the molecular interactions involving pinostrobin, pinocembrin, and the MMP enzyme. Consequently, this approach facilitates the identification of the potential of pinostrobin and pinocembrin as anti-photoaging agents.

## Methods

### Preparation and optimization of test compounds

The three-dimensional structures of the test compounds and the positive controls were retrieved from https://pubchem.ncbi.nlm.nih.gov/. The structure of the test compounds were optimized using the HyperChem 8 program with included the hydrogen atoms. The test compounds included pinostrobin and pinocembrin, while the positive control encompassed retinol and vitamin C. The structural optimization employed the Austin Model (AM1) semi-empirical computational method with single-point calculation settings and geometry optimization.

### Preparation of protein targets

The protein target structure were obtained from http://www.rcsb.org/pdb/home/home.do sourced in their the active form bound to the native ligand. The protein targets used were MMP-1 (PDB ID: 966C), MMP-3 (PDB ID: 1G4K), and MMP-9 (PDB ID: 2OW0).

### Validation of molecular docking

The validation of the molecular docking method involved the redocking of native ligands that had been previously dissociated from the target protein. For this validation process, the AutoDockTools 1.5.6 program was employed, incorporates Autodock4 and Autogrid4 functionalities. Chain selection was carried out at this stage. The validation outcome was represented by the root-mean-square deviation (RMSD) value between the native ligand and the target protein. The molecular docking process was considered valid if the RMSD value is ≤ 3 Å, indicating acceptability of the utilized method.

### Docking of test compounds on protein targets

The optimized structures of pinostrobin, pinocembrin, and positive control were subjected to docking into the prepared protein target from which the native ligand had been removed. This process employed a previously validated method. Utilizing the AutoDockTools 1.5.6 application program, integrated with Autodock4 and Autogrid4 programs, the docking procedure was conducted.

The outcomes derived from the docking process of pinostrobin, pinocembrin, and the positive control onto this protein target encompassed the bond energy value and the nature of the formed hydrogen bonds. Subsequent to this, an analysis of the attained results was undertaken.

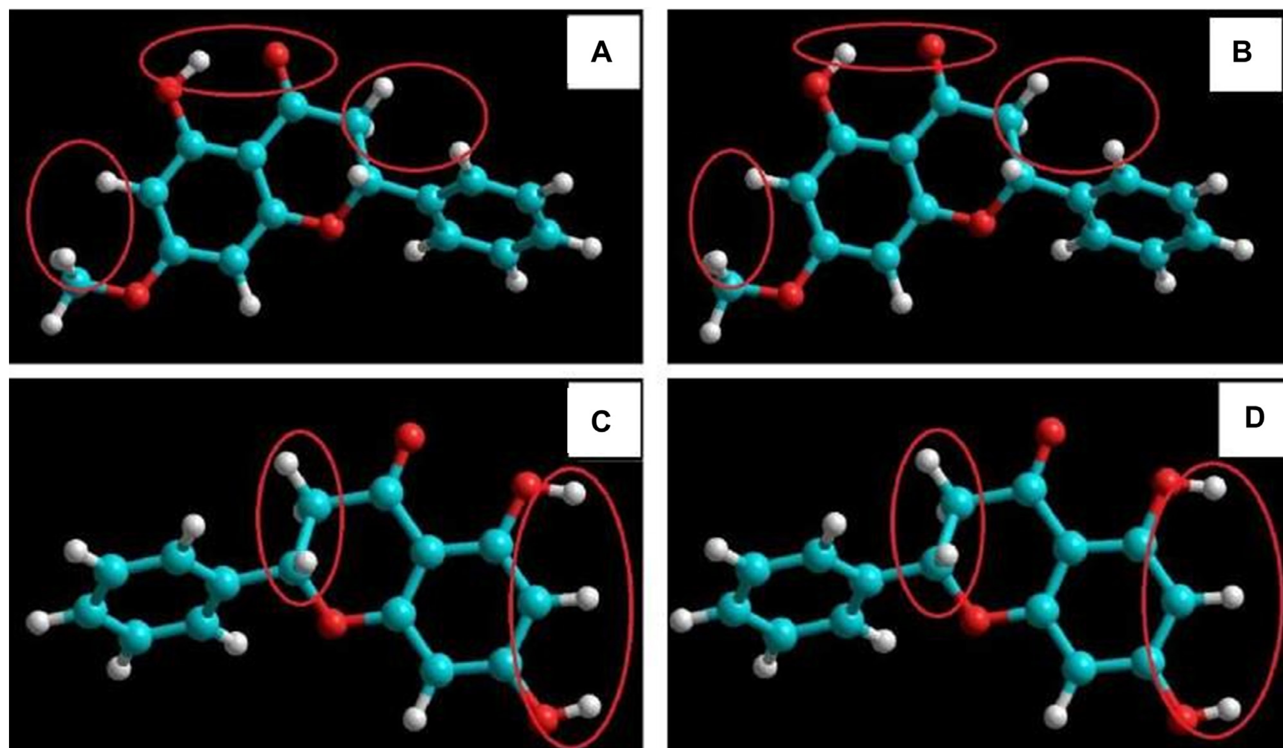


**Figure 2.** Optimization of pinostrobin compound structure a) Single point calculation b) and geometric optimization, and the optimization of pinocembrin compound structure c) Single point calculation d) and Geometric optimization. The red circle means the results after the optimization of the test compound

### Analysis data

The data were analyzed using descriptive methods. The outcomes derived from molecular docking encompassed the bond energy and the type of bond between the compound and protein target. The energy value indicates the affinity between the compound and the protein target. A more negative energy value corresponds to a stronger ligand binding affinity to a protein target.

## Results

### Preparation and structure optimization of test compounds

The successful optimization of the 3D structure of the test compounds signifies that the optimization process effectively reduces the overall energy of the compounds. This results in a stable structure with lower energy than the initial energy in single-point calculations. The outcomes of the 3D structure optimization for the positive test and control compounds are shown in Table 1, while visual representations are available in Figures 2, 3, and 4.

### Protein target preparation

Protein preparation was carried out on the 3D structure of the target protein, namely the enzymes MMP-1 (PDB ID: 966C), MMP-3 (PDB ID: 1G4K), and MMP-9 (PDB ID: 2OW0), which were downloaded from http://www.rcsb.org/pdb/home/home.do.

### Molecular docking method validation

Among the ten conformations of the native ligand within the protein target's binding site, the most optimal conformation with the lowest RMSD value was selected. This particular conformation was chosen based on its adherence to the RMSD requirement of ≤ 3 Å, signifying that the coordinates of the native ligand closely align with the initial position within the active site of the protein target [6].

### Docking of test compounds on protein targets

The docking procedure for the test compounds yielded ten interaction conformations with each target protein, namely MMP-1, MMP-3, and MMP-9. Table 3 displays the values of the most negative bond energies and the hydrogen bonds formed through docking in the

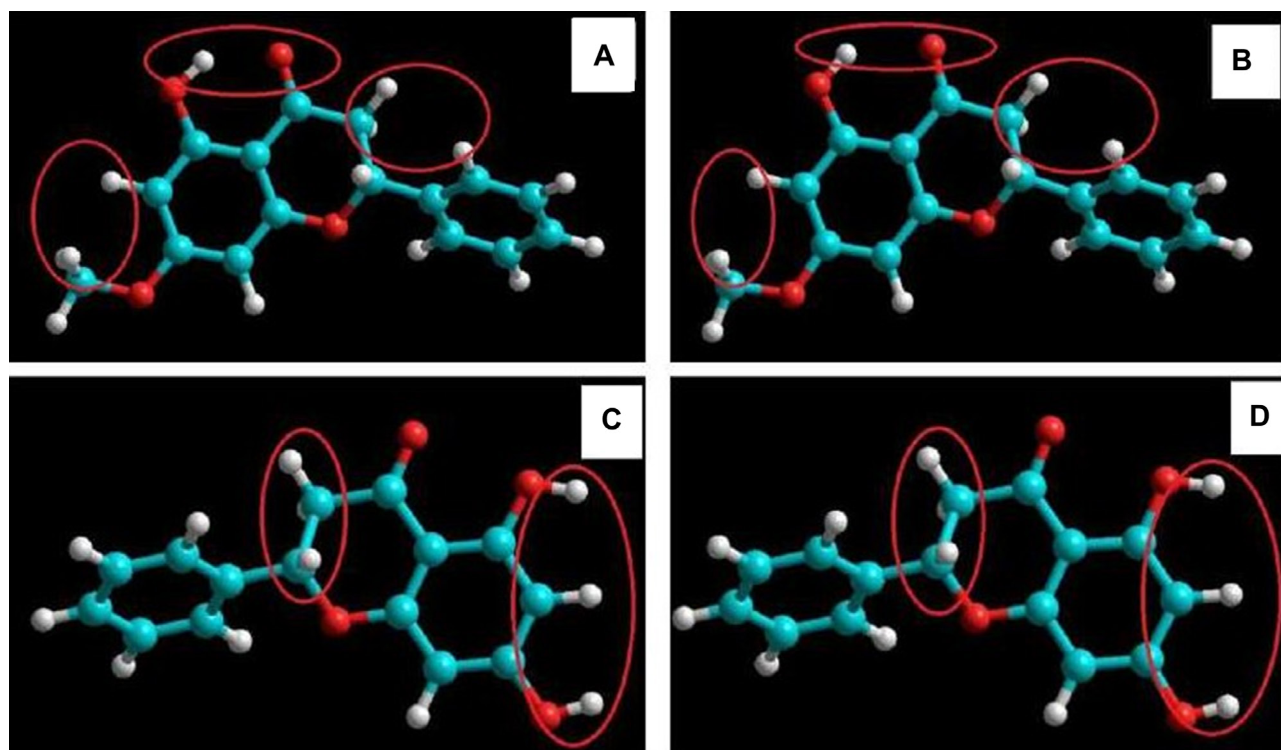


**Figure 3.** Optimization of retinol compound structure a) Single point calculation b) and Geometric optimization, and optimization of vitamin C compound structure c) Single point calculation and d) Geometric optimization. The red circle means the results after the optimization of the test compound

**Table 1.** Results of 3D structure optimization compounds

| Compounds | Total molecular energy (kcal/mol) | |
|---|---|---|
| | Single point calculation | Geometry optimization |
| Pinostrobin | -3795.91 | -3804.87 |
| Pinocembrin | -3524.62 | -3530.14 |
| Retinol | -5057.94 | -5072.28 |
| Vitamin C | -2023.93 | -2037.96 |

**Table 2.** Grid box values between native ligand and MMP-1, MMP-3, and MMP-9

| Protein | Grid Box Settings | Conformation | RMSD (Å) | |
|---|---|---|---|---|
| | Grid Size | Grid Center | | |
| MMP-1 | x = 40 | x = 9.16 | 5 | 1.00 |
| | x = 40 | y = -10.35 | | |
| | x = 40 | z = 38.39 | | |
| MMP-3 | x = 40 | x = 19.80 | 7 | 1.12 |
| | x = 40 | y = 37.10 | | |
| | x = 40 | z = 48.02 | | |
| MMP-9 | x = 40 | x = 25.57 | 8 | 0.99 |
| | x = 40 | y = 7.54 | | |
| | x = 40 | z = 47.60 | | |

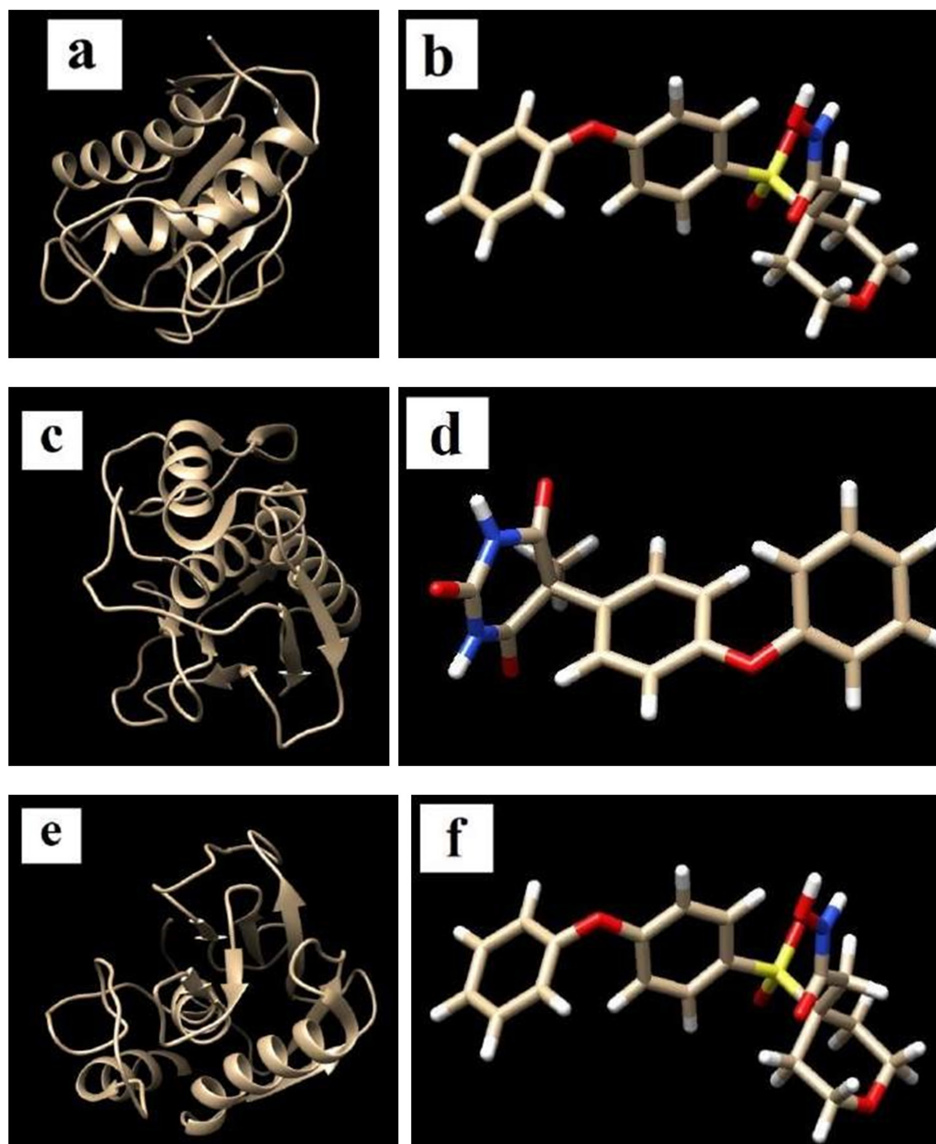


**Figure 4.** Enzyme chain structure of MMP-1, MMP-3, MMP-9 and their native ligand. (a) Chain structure of MMP-1 enzyme A; (b) Structure of native ligand RS2; (c) MMP-3 enzyme A chain structure; (d) Structure of native ligand HQQ; (e) MMP-9 enzyme A chain Structure; (f) Structure of the 6MR native ligand

chosen conformations of pinostrobin, pinocembrin, and the positive control compounds with their corresponding target proteins, MMP-1, MMP-3, and MMP-9.

## Discussion

The test compounds underwent optimization through the AM-1 semi-empirical method, employing the Hyperchem 8 application. The optimization of the test compound's 3D structure consisted of two stages: single-point calculations and geometry optimization. The success of the 3D structure optimization for the test compounds was affirmed by the outcome of the geometry optimization, which effectively minimized the total energy of the compounds. This success resulted in attaining a stable structure with lower energy levels than the initial energy in the single-point calculations.

The process of preparing the protein involved several steps. Firstly, a chain was chosen from the target protein to facilitate the identification of the binding site's coordinate space for ligand docking. This selection was guided by information about the binding site of the native ligand, which possesses inhibitory activity and serves as an organic compound within the protein. In cases where the target protein has multiple chains all binding to the native ligand, any one of

**Table 3.** Comparison of bond energy and interaction models of ompound on protein target

| Protein target | Ligand | Binding energy (kcal/mol) | amino acid residues | Groups in hydrogen bonds (protein-ligands) |
|---|---|---|---|---|
| **MMP-1** | Native Ligand | -10.74 | Asn73 Leu74 Ala75 Tyr133 | HN-O25 HD22-O25 HN-O26 HN-O31 |
| | Pinostrobin | -8.59 | Thr134 | O-HO |
| | Pinocembrin | -8.69 | His111 | HE2-O |
| | Retinol | -9.69 | Asn73<br>Leu74 | HD22-O<br>HN-O |
| | Vitamin C | -5.43 | Leu128 | O-HO |
| | | | Tyr130 | O-HO |
| | | | Ser132 | O-HO |
| | | | Thr134 | HG1-O |
| **MMP-3** | Native | -10.43 | Leu82 | HN-O6 |
| | Ligand | | Ala83 | HN-O6 |
| | | | His119 | HE2-O2 |
| | | | Glu120 | OE2-HN |
| | | | His123 | HE2-O2 |
| | | | His129 | HE2-O2 |
| | Pinostrobin | -9.45 | - | - |
| | Pinocembrin | -9.74 | - | - |
| | Retinol | -10.66 | Leu82 | HN-O |
| | Vitamin C | -5.63 | Leu115 | O-HO |
| | | | His142 | O-HO |
| **MMP-9** | Native | -10.68 | Leu79 | HN-O |
| | Ligand | | Ala80 | HN-O |
| | | | His117 | HE2-OAP |
| | | | Gln118 | HE21-OBE |
| | | | His121 | HE2-OAP |
| | | | His127 | HE2-OAP |
| | Pinostrobin | -9.25 | - | - |
| | Pinocembrin | -9.63 | Pro137 | O-HO |
| | Retinol | -10.54 | Leu79 | HN-O |
| | | | Ala80 | HN-O |
| | | | | O-HO |
| | Vitamin C | -1.8 | - | - |

Note:
The number after the amino acid residue indicates the sequence in the protein's amino acid chain; Example: Asn73 (73rd order aspargine amino acid residue); Ala (Alanin); Asn (Aspargin); Glu (Glutamate); Gln (Glutamine); His (Histidine); Leu (Leucine); Pro (Proline); Ser (Serine); Thr (Threonine); and Tyr (Tyrosine); HD22-Ox (Hydrogen atom (H) position D (delta) number 22 on the amino acid residue binds to the oxygen atom (O) number x on the ligand); O-HO (Oxygen atoms (O) on amino acid residues bond with hydrogen atoms (H) oxygen branches on ligands); D (deltas); E (Epsilon); G (Gamma); N (nitrogen branch)

those chains can be randomly selected. For the MMP-1 enzyme, only the A chain was present, making it the logical choice for MMP-1 protein preparation. The native ligand associated with the A chain, known as N-hydroxy2-[4-(4-phenoxy-benzene sulfonyl)-tetrahydro-pyran-4-yl]-acetamide (ID: RS2), operates as an inhibitor of the MMP-1. The MMP-3 consists of three chains: A, B, and C. Among these, chain A was selected due to its association with the native ligand (ID: HQQ), demonstrating inhibitory activity. This ligand is present in all three chains. Similarly, for the MMP-9, which comprises chains A and B, the decision was made to choose chain A. Both chains contained a native ligand with inhibitory properties, specifically N-[(4'-iodobiphenyl4-yl)sulfonyl]-d-tryptophan (ID: 6MR). As part of the preparation process, water molecules ($H_2O$) were removed to prevent potential disruption and elongation of the docking simulation.

The docking process employed the Lamarckian Genetic Algorithm (LGA) output parameters. Based on the obtained results, it is evident that the utilized method is valid, as it fulfills the validation criterion of ≤ 3 Å. Notably, all test compounds exhibited negative bond energies, indicating that both pinostrobin and pinocembrin have an affinity for the target proteins MMP-1, MMP-3, and MMP-9. The observed bond interactions are predominantly hydrogen bonds. These hydrogen bonds form through interactions between amino acids at MMP-1, MMP-3, and MMP-9 binding sites and specific groups within the test compounds acting as hydrogen donors or acceptors based on their electronegativity. Notably, the strength of these hydrogen bonds typically falls within the range of approximately 3-7 kcal/mol [7]. Visualized results highlight that MMP-9 did not form hydrogen bonds with vitamin C or pinostrobin. Similarly, pinostrobin and pinocembrin did not engage in hydrogen bond formation within MMP-3. This outcome suggests that the interaction between vitamin C and pinostrobin with MMP-9, as indicated by the bond energy obtained, did not primarily involve the creation of hydrogen bonds.

This affinity indicates that the test compounds can help slow down collagen breakdown by inhibiting MMP-1, MMP-3, and MMP-9. This action can potentially reduce skin photoaging. When comparing the docking results of pinostrobin and pinocembrin with the native ligand and the control for MMP-1, MMP-3, and MMP-9, it is observed that the bond energy of pinostrobin and pinocembrin is not more negative than the bond energy of the native ligand in these three proteins. It means that the tested compound's affinity is not stronger than the native ligand in terms of binding energy for MMP-1, MMP-3, and MMP-9. Comparing the results to the vitamin C, the bond energy of pinostrobin and pinocembrin is more negative than vitamin C for the three target proteins, suggesting that pinostrobin and pinocembrin have more affinity for binding to MMP-1, MMP-3, and MMP-9. This indicates that pinostrobin and pinocembrin have a stronger affinity than vitamin C in their interactions with MMP-1, MMP-3, and MMP-9.

Lutein has demonstrated its effectiveness as an anti-photoaging agent by inhibiting the MMP-1 protein through a specific mechanism [8]. The study's findings revealed that lutein exhibits an affinity for MMP-1, evident from its binding energy of -12.28 kcal/mol, which is notably lower than the binding energy of the MMP-1 protein's native ligand RS2 (-10.83 kcal/mol). Furthermore, lutein engages with the MMP-1 protein through hydrogen bonding, specifically with the His228 residue. This hydrogen bonding interaction mirrors the hydrogen bonds formed between the RS2 native ligand and the MMP-1 protein. Lutein's primary photoprotective mechanism stems from its possession of conjugated double bonds within its structure. This unique configuration allows it to absorb potentially harmful light, including UV radiation. In a separate in silico study involving astaxanthin, derived from the radio-resistant bacterium Deinococcus sp. strain WMA-LM9, it was observed that this carotenoid exhibits a strong binding affinity to the MMP-1 protein, as indicated by its binding energy of -10.5 kcal/mol. Additionally, an in vivo study focusing on a 16-week supplementation regimen involving mixed carotenoids (β-carotene and lycopene) and proanthocyanidins demonstrated the ability to reduce the UV-induced expression of MMP-1.

## Conclusion

The pinostrobin and pinocembrin compounds have an affinity for the target protein MMP-1, MMP-3, and MMP-9, as shown by the negative binding energy. The affinity of pinostrobin and pinocembrin was weaker than retinol, but stronger than vitamin C. We also found that hydrogen bonding is not the only form of protein-ligand interaction that can occur. Pinostrobin and pinocembrin still have potential as anti-photoaging agents. However, other applications, such as Molegro Virtual Docking, should be used to

obtain visualization results of bonds and interactions other than the hydrogen bonds formed between the test compounds with the proteins, including Van der Waals interactions, hydrophobic bonds, and electrostatic bonds.

## Acknowledgment

We express our gratitude to the Department of Pharmacy, the Udayana University for providing the necessary facilities to complete this work.

## Declaration of interest

The authors declare no conflict of interest.

## Author contributions

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

Received: 26 June 2023
Revised: 14 August 2023
Accepted: 24 August 2023
Published online: 24 August 2023

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