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Research Article

In-silico Prediction of *Epigallocatechin-3-Gallate* (EGCG) vs Retinol in Photoaging Therapy

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ABSTRACT

Background: Skin aging is a cumulative damage that occurs due to complex biological processes from genetic and environmental factors that are evident in individual's appearance. Clinically photoaging causes wrinkling, telangiectasia, dryness, pigment changes and loss of elasticity. As the predominant element found in green tea, epigallocatechin-3-gallate (EGCG) exhibits an active physiological function observed in both human and animal skin. Exposure to the two components of solar UV radiation that reach the earth surface, UVA (320–400nm) and UVB (290–320nm), leads to protein oxidative damage, lipid oxidation, DNA chain damage, and depletion of antioxidant enzymes. Since 1984, all-trans retinol has been incorporated into over-the-counter (OTC) cosmetic products, yet its potential in treating photoaging continues to be investigated. **Methods:** Search Profile EGCG, Retinol, Hyaluronan, and then Bioactive Prediction with SAR. Predicted EGCG targets were analyzed using Comparative Toxicogenomics Database. Compound Profile Similarity with Tanimoto Similarity. Using AlphaFold model, we obtained three-dimensional configuration of Hyaluronan Synthase 1, as designated target protein in this study, from Uniprot database (https://www.uniprot.org/) with identifier Q92839. **Results:** Based on SAR analysis to predict potential bioactivity, it shows that EGCG has better potential than retinol as an antioxidant and free radical scavenger. Target prediction with CTD shows that in curated studies the EGCG CTD is able to target COL1A1, HAS1, NFE2L2, and MMP1. Based on tanimono similarity, the similarity between EGCG and Hyaluron is higher than Hyaluron and Retinol. **Conclusions:** Docking analysis shows that it is predicted that EGCG is better at interacting with HAS1 and MMP1.
Keywords: EGCG, docking, HAS, MMP1.

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INTRODUCTION

Skin aging is a cumulative damage that occurs due to complex biological processes from genetic and environmental factors that are evident in an individual's appearance. increasing one's age. Extrinsic skin aging type or photoaging is skin aging caused by environmental elements like exposure to UV (Ultraviolet) and sunlight radiation.[1] Clinically photoaging causes wrinkling, telangiectasia, dryness, pigment changes and loss of elasticity.[2, 3] Photoaging tends to be more pronounced within the demographic possessing skin types I, II, and III on the Fitzpatrick scale, unlike those exhibiting skin types IV, V, and VI. Among participants younger than 30 years in an Australian study, 72% of men and 47% of women exhibited moderate to severe signs of photoaging. In the darker-skinned population, wrinkles do not appear until age 50 and are less pronounced in severity than in the lighter-skinned population of the same age.[3, 4]

Plant extracts are rich in antioxidant compounds that can provide a protective shield against both UV damage and the aging process for the skin. Green tea (*Camellia sinensis*), rich in catechins, stands out as one of the plant extracts suitable for preventing the aging process. Specifically, the predominant element found in green tea, epigallocatechin-3-gallate (EGCG), exhibits an active physiological function that has been verified in both animal and human skin. Exposure to the two components of solar UV radiation that reach the earth surface, UVA (320–400 nm) and UVB (290–320 nm), leads to leads to protein oxidative damage, lipid oxidation, DNA chain damage, and depletion of antioxidant enzymes. Treating the skin of mice with EGCG has been discovered to safeguard the skin's UV-induced immune system, diminish the inflammatory reaction triggered by exposure to sunlight, and inhibit the photoaging process of the skin.[5]

Damayanti et al.'s study in rats revealed the photoaging prevention effect of EGCG through its interaction with the Keap1-Nrf2 protein, a factor that contributes to the development of photoaging. Epigallocatechin-3-gallate binds to Keap1-Nrf2, with good pharmacokinetics because it is well absorbed, and minimal to non-existent toxicity.[6]

Studies by Kim et al in Korea regarding the effects of EGCG on several mechanisms of skin protection from photoaging, namely increasing skin hydration, the parameters of which include the expression of hyaluronidase (HYAL) and hyaluronic acid syntase (HAS) gene, and increasing the expression of filagrin (FLG), transglutaminase-1, HAS -1, and HAS-2, which affect the formation of natural moisturizing factor (NMF). Epigallocatechin-3-gallate has an anti-apoptotic effect, by reducing melanin secretion and production in melanoma cells and caspase-8 and -3 in HaCaT cells.[7]

Part of the endogenous natural retinoid family, all-trans retinol, also recognized as Vitamin A alcohol, functions as a precursor for the synthesis of retinal and retinoic acid within the body. Despite being used in over-the-counter (OTC) cosmetic products since 1984, the therapeutic potential of all-trans retinol for

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Table 1: Docking coordinates.

Macromolecule	Center			Dimension (Angstrom)		
	x	y	z	x	y	z
MMP1	70.2485	11.7335	13.9065	20	20	20
HAS1	-1.9947	-2.842	-0.6515	25	25	25

treating photoaging became evident through studies. Kang et al. (1995) demonstrated that the application of this substance to normal human skin leads to an increase in mRNA expression of retinoic acid, CRBP proteins, and CRABP II, as well as epidermal thickening. Notably, this study found that, in comparison to tretinoin, retinol exhibited minimal symptoms of erythema and irritation. In a separate 14-day study involving six participants, Fluhr et al. (1999) confirmed that retinol resulted in significantly reduced transepidermal water loss, erythema, and scaling. Fisher et al. (1996, 1997) further supported these findings by revealing that retinol inhibits UV-induced MMPs and encourages the production of collagen in skin undergoing photoaging.[8]

The potential antiaging therapy of EGCG and retinol in this study was compared by looking at protein-drug interactions, especially in the hyaluronide and MMP 1 binding.

MATERIALS AND METHODS

Search Profile EGCG, Retinol, Hyaluronan

The simplified molecular-input line-entry system (SMILE) and 3D structures of the ligands slated for analysis were obtained from the PubChem database at https://pubchem.ncbi.nlm.nih.gov/ (refer to Table 1). SMILE is a unique code that describes the structure and profile of the ligand to be analyzed.

Bioactive Prediction with SAR

The SMILE obtained from PubChem was then analyzed using the Structure Analysis Relationship approach to predict its potential as an antiaging agent. SAR predictions were made utilizing the Way2Drug Pass Online webserver at http://www.way2drug.com/passonline/.

EGCG & Retinol Target Prediction

Predicted EGCG targets were analyzed using the Comparative Toxicogenomics Database (http://ctdbase.org/voc.go?type=chem). The CTD database contains the effect of giving a compound on the expression of certain mRNAs & proteins that have been published in the CTD database curated journal.

Compound Profile Similarity with Tanimoto Similarity

The search for similarity between EGCG, Retinol, and Hyaluronan was analyzed using the Tanimoto Similarity approach to RDkit.

Molecular docking

Obtained from the Uniprot database (https://www.uniprot.org/), the 3D structure of the selected target protein, Hyaluronan Synthase 1, was modeled utilizing Alphafold with ID Q92839 Meanwhile, MMP1 uses PDB ID 2TCL (THE CATALYTIC DOMAIN OF HUMAN FIBROBLAST COLLAGENASE COMPLEXED WITH AN INHIBITOR) (https://www.rcsb.org/). Furthermore, the 3D structure of each ligand was obtained from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov). Then, the protein was prepared by eliminating water molecules in PyMol 2.3.1 software. Simultaneously, the ligands underwent energy minimization utilizing the universal force field in Open Babel within Pyrx v.0.9.9 software. Docking procedures were conducted through Autodock Vina integrated in Pyrx v.0.9.9 (Trott & Olson, 2010). The targeted docking method was employed in this process with an exhaustiveness parameter set to 50. Adjustments to the grid box size were made to correspond with the positions of amino acid residues based on predictions of binding sites utilizing PrankWeb (https://prankweb.cz/) (Dávid et al., 2022). The disclosed binding affinity or affinity energy from the docking results elucidates the interaction between the compound and the protein. Moreover, the visualization of interactions between the compounds and the proteins they docked with was conducted through BioVia Discovery Studio 2021 software.

RESULT

Bioactive Prediction with SAR

Analysis using the SAR approach shows that EGCG has better potential than retinol (Figure 1) as:

- Hyaluronic acid agonists
- NFKB transcription factor inhibitors
- Antioxidants
- Free radical scavengers

SAR is an approach by comparing the input compound profile with the database. The more similar the structure, the more confidence the resulting value. The parameter used is the Pa Score Prediction with a value range of 0 – 1. If the predicted score has a value > 0.7 then the predictive value is strong, because the profile similarity between the input compounds and the database is high.[9-11]

EGCG & Retinol Target Predictions

EGCG and Retinol target predictions were analyzed using the CTD database. In the database it is recorded that EGCG has the following roles (Table 2):

Compound Profile Similarity with Tanimono Similarity

The similarity between EGCG and Hyaluron is higher than that of Hyaluron and Retinol. Although both scores are still relatively low (Table 3). Tanimono Similarity has a score range of 0 – 1. The closer it is to 1, the more similar the two structures are.

Molecular Docking is carried out targeted site (specific site) docking by looking at the position of the binding control or prediction of the binding cavity of the analyzed protein. For MMP1, the docking

Table 2: EGCG target predictions.

Compound	Target	Description
epigallocatechin gallate	COL1A1	epigallocatechin gallate leads to an **increase** in COL1A1 mRNA expression
epigallocatechin gallate	HAS1	epigallocatechin gallate leads to a **decrease** in HAS1 mRNA expression
epigallocatechin gallate	MMP1	epigallocatechin gallate **inhibits** the reaction [IL1B protein leads to an increase in MMP1 mRNA expression]
epigallocatechin gallate	MMP1	epigallocatechin gallate **inhibits** the reaction [Particulate Matter leads to an increase in MMP1 protein expression]
epigallocatechin gallate	NFE2L2	epigallocatechin gallate leads to an **increase** in NFE2L2 mRNA expression
epigallocatechin gallate	NFE2L2	epigallocatechin gallate leads to an **increase** in NFE2L2 protein expression

Table 3: Similarity of EGCG & Retinol with Hyaluron.

Compound	Tanimoto Similarity Score
(-)-Epigallocatechin gallate	0.091
Retinol	0.068

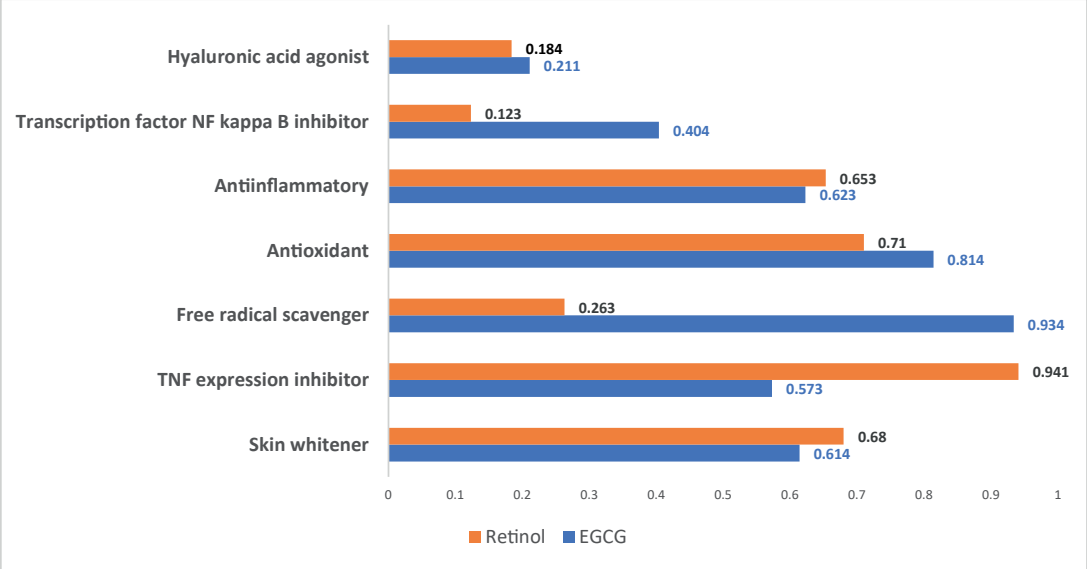

Figure 1. Prediction of Bioactive Retinol and EGCG Potency.

Figure 2. Visualization of HAS1 Docking Results. A) Gray (HAS1), Orange (Hyaluron), Blue (Retinol), Green (EGCG). B) Wheat (MMP1), Red (Control Inhibitor), Blue (Retinol), Green (EGCG).

Table 3: Similarity of EGCG & Retinol with Hyaluron.

Compound	Tanimoto Similarity Score
(-)-Epigallocatechin gallate	0.091
Retinol	0.068

Table 4: Prediction of Binding Affinity with Molecular Docking Approach.

Macromolecule	Ligand	Binding Affinity (kcal/mol)	RMSD (Angstrom)
HAS1	Hyaluron	- 10.2	1.825
	EGCG	- 9.6	
	Retinol	- 8.0	
	Ro-31-4724	- 7.3	
MMP1	EGCG	- 7.6	
	Retinol	- 6.9	

coordinates are directed to the control Ro-31-4724 position. Meanwhile for HAS1, because there is no complex agonist yet, it is predicted that the binding site will use PrankWeb with a probability of 0.99 (range 0 – 1):

A_102 A_104 A_106 A_215 A_237 A_238 A_239 A_267 A_281 A_284 A_285 A_288 A_289 A_292 A_293 A_296 A_303 A_304 A_306 A_308 A_336 A_337 A_338 A_339 A_340 A_344 A_363 A_364 A_366 A_371 A_375 A_376 A_378 A_379 A_380 A_383 A_386 A_387 A_390 A_401 A_404 A_405 A_408 A_464 A_468 A_472 A_476 A_488 A_490 A_491 A_493.

The validation of docking results can be reviewed from various sides

1. RMSD (Root mean standart deviation) value by comparing redock results and crystallographic results. Docking results that can be used must be less than < 2 Angstroms (Table 4)
2. The docking positions between the comparison and control ligands are in the same cavity (Figure 2).
3. The binding affinity residues were the same between the control and the comparison ligands (Table 5).

Based on docking analysis, hyaluron (-10.2 kcal/mol) is the best ligand to bind to HAS1, followed by EGCG docking results (-9.6 kcal/mol). The more negative the binding affinity value, the more favorable the bond will be (the stronger the prediction for binding to that position). As for MMP1, EGCG can bind at a position similar to the MMP1 inhibitor in previous research (2TCL) (Table 4).

Based on the docking analysis with HAS1, hyaluron, EGCG, and retinol are able to interact with the input amino acid residues from the PrankWeb prediction results. Hydrophobic interactions contribute to enhancing

Table 5: Interactions of Macromolecular Amino Acid Residues and Test Ligands.

Macromolecule	Ligand	Interaction Bond		
		Hydrogen	Hydrophobic	Others
HAS1	Hyaluron	TYR285 ARG293 GLN296 SER408 SER303 GLU292 CYS304 ARG340 ASP339 ARG378 TRP379		ARG284 (Unfavorable Bond)
	EGCG	**TYR285** SER491 GLU106	PRO308	ARG493 (Electrostatic) ASP237 (Electrostatic) **ARG284** (Unvaforable Bond)
	Retinol		LEU472 TYR383 **TRP379** ALA288 **TYR285** VAL267 PRO308 PHE289	
	Control	GLY79 TYR140 PRO138 HIS118 HIS128 GLU119 HIS122 ALA82 LEU81	TYR110	
MMP1	EGCG	ASN80 **HIS128 HIS118** ALA84 SER72 HIS83	**HIS122**	**GLU119** (Electrostatic) GLN86 (Unfavorable Bond) ASN 6 (Unfavorable Bond)
	Retinol	**HIS118** SER139	**HIS122** VAL115 **LEU81** HIS83 HIS128	ARG114 TYR137

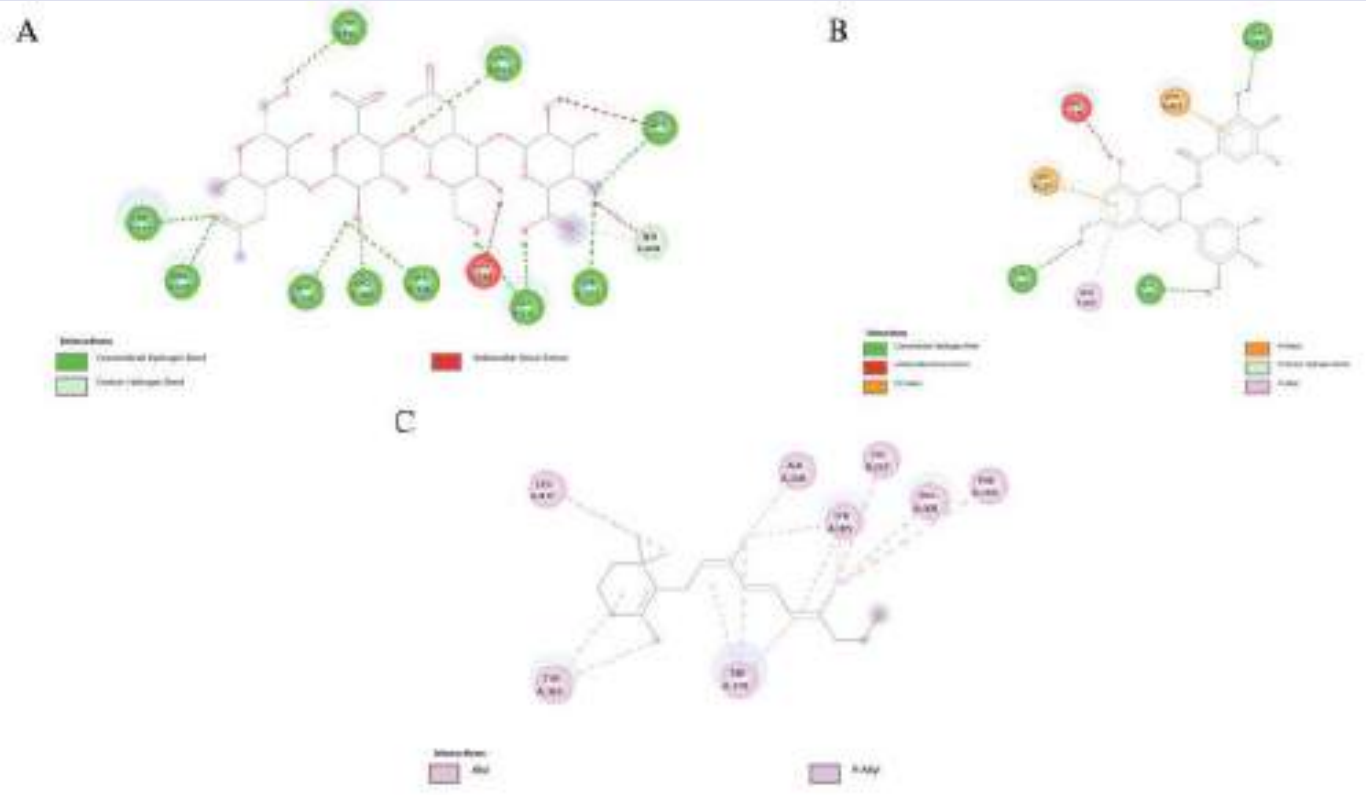

Figure 3. Interaction of Amino Acid Residues with HAS1 a) Control, b) EGCG, c) Retinol

the bonds between macromolecules and ligands, ensuring stability in the formed bonds (Table 5, Figure 3). Meanwhile, analysis with MMP1 showed that there were 4 residues that were the same as EGCG and control, and 3 residues that were the same between retinol and control. EGCG and Control have more similar residues than retinol in targeting MMP1.

DISCUSSION

The result of this *in silico* study demonstrated Based on SAR analysis to predict potential bioactivity, it shows that EGCG has better potential than retinol as an antioxidant and free radical scavenger. Target prediction with CTD shows that in curated studies the EGCG CTD is able to target COL1A1, HAS1, NFE2L2, and MMP1. Based on tanimono similarity, the similarity between EGCG and Hyaluron is higher than that of Hyaluron and Retinol. However, their scores are still relatively low. Expression of hyaluronic acid syntase (HAS) and hyaluronidase (HYAL) genes, and increases the expression of filagrin (FLG), transglutaminase-1, HAS-1, and HAS-2, which affect the formation of natural moisturizing factor (NMF).[8]

Photoaging occurs due to changes in the MAPK signaling pathway, manifested as an increased JNK and p38 pathways and a decreased ERK-dependent-MAPK, leading to decreased cell proliferation, cell differentiation, and cell defense. The activation of the MAPK pathway during photoaging leads to an elevation in c-Jun and c-Jun N-terminal kinase levels.

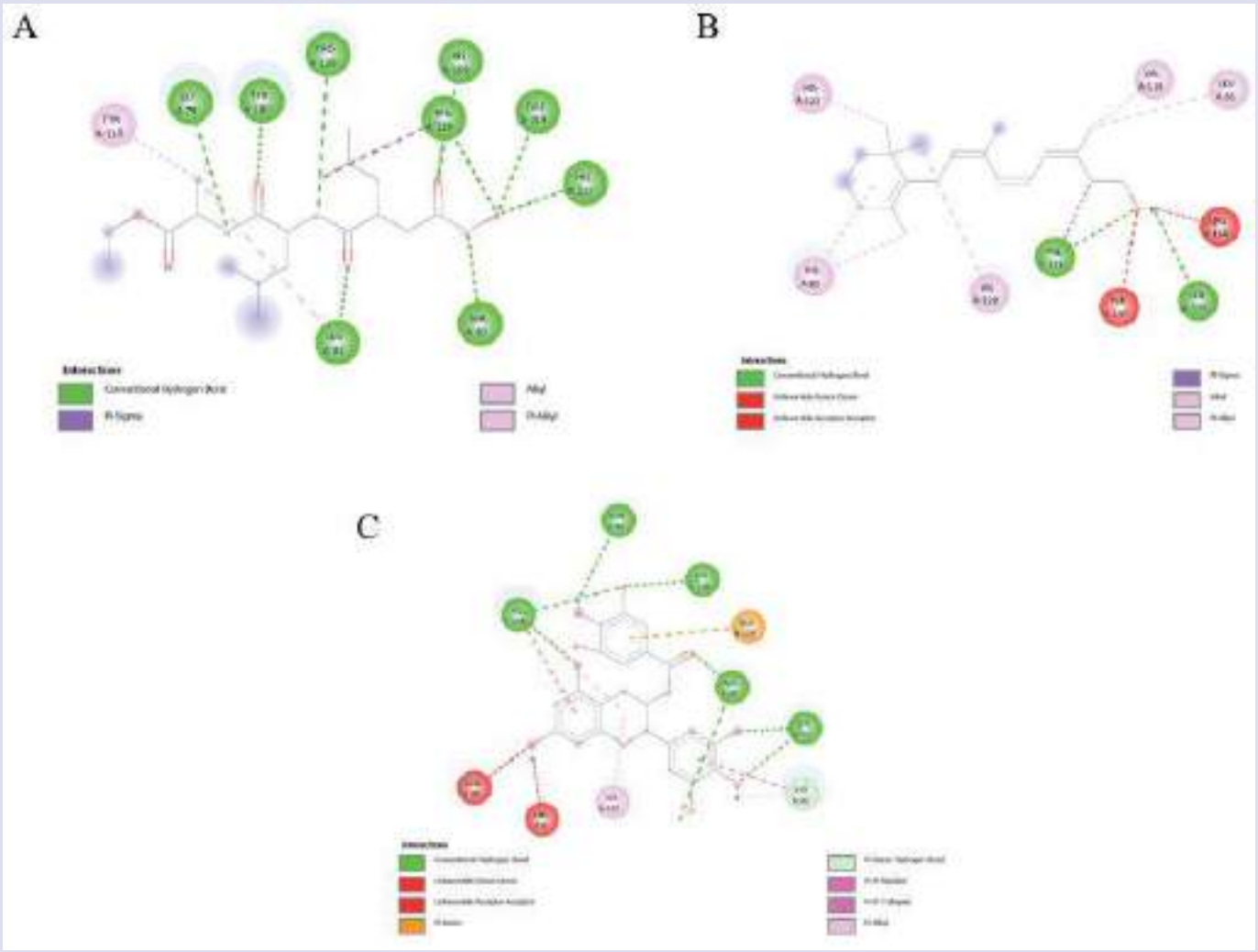

Figure 4. Interaction of Amino Acid Residues with MMP1 a) Control, b) EGCG, c) Retinol.

As the c-Jun protein is a component of the AP-1 transcription factor, an increase in c-Jun will consequently lead to an elevation in AP-1. The AP-1 upgrade will increase MMP, especially MMP 9 (gellatinase B), MMP-3 (stromelysin 1), and MMP-1 (collagenase 1 or interstitial collagenase); and reduce expression tissue inhibitors MMP or TIMP. Matrix metalloproteinase-1 (MMP-1) increase collagen types I and III degradation. Skin aging, especially photoaging, causes collagen loss fragmented due to MMP enzyme activation, increased ROS production in mitochondria, and increased oxidative stress, which increases activation cytokines and growth factor (GF) receptors in keratinocytes and fibroblasts. Various polyphenols in green tea, including EGCG, have ROS scavenging activity, making them good candidates for photoaging prevention.[2]

A study conducted by Damayanti et al at Airlangga University, Indonesia found that EGCG has a photoaging prevention effect through its interaction with the Keap1-Nrf2 protein contributing to the development of photoaging. Epigallocatechin gallate binds to Keap1-Nrf2, with good pharmacokinetics because it can be well absorbed, and minimal to no toxicity.[6] EGCG also prevents damage to DNA, especially in the epidermis. Initial damage to the skin is shown by the skin color which becomes erythematous, and EGCG plays a direct role in reducing erythema on the skin after exposure to UV light.[6, 12]

CONCLUSION

Docking analysis showed that it was predicted that EGCG was better at interacting with HAS1 and MMP1.

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