ANTIHYPERPIGMENTATION POTENTIAL OF PERSIMMON VINEGAR: A NETWORK PHARMACOLOGY AND MOLECULAR DOCKING APPROACH

Fitriyani[1], Fajar Prayoga[2], M. Abbas Ar-riziq[2], Roihatul Mutiah[2*]

[1]Master Program in Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, East Java, Indonesia
[2]Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim, Malang 65151, East Java, Indonesia

Email: roiha@farmasi.uin-malang.ac.id

Abstract

Hyperpigmentation is a condition characterized by increased melanin production, prompting the search for natural agents with potential skin-brightening effects. Persimmon vinegar contains various bioactive compounds that may modulate melanogenesis pathways. This study aimed to predict the antihyperpigmentation potential of bioactive compounds in persimmon vinegar using network pharmacology and molecular docking approaches. Network pharmacology analysis was conducted using GeneCards, DisGeNET, STRING, and Cytoscape to identify interactions between active compounds and hyperpigmentation-related targets, while molecular docking was performed using AutoDockTools against tyrosinase (PDB: 2Y9X). A total of 386 target proteins were identified, with four overlapping targets (SLC29A3, AHR, COL7A1, and MFN2) associated with hyperpigmentation pathways. These targets were linked to three major active compounds: aspartic acid, gallic acid, and malic acid. Molecular docking analysis showed that all three compounds interacted with residues within the tyrosinase active site, including HIS259, ASN260, and HIS263. Among the tested compounds, gallic acid exhibited the lowest binding energy (−1.52 kcal/mol), although its affinity remained weaker than the native ligand (−4.92 kcal/mol). Overall, the findings suggest that persimmon vinegar contains bioactive compounds with potential relevance to antihyperpigmentation mechanisms and may serve as candidates for further experimental investigation.

Keywords: *Hyperpigmentation; Molecular docking; Network pharmacology; Persimmon vinergar; Tyrosinase*

Introduction

Hyperpigmentation is a dermatological condition characterized by discoloration of the skin, causing it to appear darker or uneven in tone (Moolla and Miller-Monthrope, 2022). Several major factors contribute to hyperpigmentation, including sun exposure, which stimulates excessive melanin production; genetic factors that determine the number and activity of melanocytes; and hormonal changes such as estrogen and progesterone fluctuations, which often trigger melasma in pregnant women or users of oral contraceptives. In addition, inflammation or skin injuries resulting from acne, eczema, or burns can lead to post-inflammatory hyperpigmentation. The use of certain medications,

such as antibiotics and antimalarials, is also known to increase skin sensitivity to UV radiation. With advancing age, the remaining melanocytes tend to become more active, leading to the appearance of age spots (Thawabteh *et al.*, 2023). According to Nautiyal and Wairkar (2021) treatment options for hyperpigmentation include topical, oral, procedural, and innovative therapies. Topical therapies utilize agents such as hydroquinone and retinoids to inhibit melanin synthesis. Oral therapies like tranexamic acid and glutathione work systemically from within the body. Procedural methods such as chemical peels and laser therapy help remove excess pigment. However, these treatments may cause mild to severe side effects, making herbal remedies derived from traditional plants a potentially effective, natural, and low-risk alternative for managing hyperpigmentation (Rathee *et al.*, 2021).

Persimmon vinegar has gained increasing attention as a natural antihyperpigmentation agent due to its rich content of bioactive compounds with strong antioxidant properties. It contains various active constituents such as carotenoids, proanthocyanidins, and gallic acid, which exhibit potent antioxidant activity and can inhibit melanin production in the skin (Direito *et al.*, 2021). These compounds act by reducing oxidative stress and inhibiting tyrosinase, the key enzyme responsible for melanin synthesis. Additionally, the organic acids present in persimmon vinegar contribute to the exfoliation process, helping remove dead skin cells and brighten hyperpigmented areas. With its natural composition and minimal side effects, persimmon vinegar has significant potential as a safe and effective alternative treatment for hyperpigmentation (Matheus *et al.*, 2022).

The network pharmacology approach can be used to understand how the active compounds in persimmon vinegar act on biological pathways associated with hyperpigmentation. This approach is particularly valuable because natural compounds often exhibit complex effects on multiple biological targets (Xu *et al.*, 2019). The use of this method aims to identify key targets and pathways influenced by these compounds and to provide a more comprehensive understanding of their underlying mechanisms.

In addition to network pharmacology, the molecular docking approach is essential for predicting the direct interactions between persimmon vinegar's bioactive compounds and key proteins involved in melanogenesis, particularly tyrosinase. Molecular docking allows for the analysis of binding energy, ligand positioning within the active site, and identification of crucial residues involved in enzyme inhibition. This method has been widely used to validate the potential of phenolic compounds as tyrosinase inhibitors and melanogenesis regulators, thereby reinforcing network pharmacology findings and offering mechanistic support prior to conducting in vitro or in vivo studies. Thus, integrating molecular docking ensures that the active compounds in persimmon vinegar possess biologically relevant affinity toward antihyperpigmentation targets (Nazir *et al.*, 2022).

Method

This study is an *in silico* investigation consisting of two main stages, network pharmacology analysis and molecular docking. In the network pharmacology stage, the bioactive compounds in persimmon vinegar were identified, followed by the

determination of target proteins involved in the hyperpigmentation process. This analysis aimed to map the relationships between compounds, target proteins, and relevant biological pathways, thereby providing a systemic understanding of the potential antihyperpigmentation mechanisms. The subsequent stage, molecular docking, was conducted to predict the binding affinity and interaction modes of these bioactive compounds with key proteins involved in melanogenesis, particularly tyrosinase as the primary enzyme responsible for melanin formation. The integration of these two *in silico* approaches offers a comprehensive mechanistic insight into the potential of persimmon vinegar as a natural antihyperpigmentation agent.

The tools used for the network pharmacology analysis included GeneCards (https://www.genecards.org), Cytoscape v3.10.1 (https://cytoscape.org), DisGeNET (https://www.disgenet.org), and STRING (https://www.string-db.org). Meanwhile, the tools employed for the molecular docking analysis consisted of AutoDockTools 1.5.6 (https://autodock.scripps.edu), the RCSB Protein Data Bank (PDB) (https://www.rcsb.org), PubChem (https://pubchem.ncbi.nlm.nih.gov), PyMOL (https://pymol.org), and Discovery Studio Visualizer (DSV) (https://discover.3ds.com/discovery-studio-visualizer-download).

Network Pharmacology

The network pharmacology analysis was conducted to map the active compounds, target proteins, and biological pathways related to melanogenesis. Identification of the bioactive compounds was carried out through a literature review using articles indexed in Google Scholar and Scopus (Stracke and Trisolini, 2021). Target proteins associated with hyperpigmentation were then retrieved from GeneCards using a relevance score threshold of ≥10 (Lin and Hu, 2021; Tjandrawinata *et al.*, 2022) and further validated through DisGeNET, a database of gene–disease associations (Rosyadah, Afendi and Kusuma, 2017). The compound–target interaction network was analyzed and visualized using Cytoscape v3.10.3 (Piñero *et al.*, 2021), while protein–protein interactions were examined using STRING with a minimum interaction score of 0.400 to identify the most influential key genes (Noor *et al.*, 2022). Subsequently, Gene Ontology (GO) and KEGG pathway analyses were employed to categorize the biological functions and molecular pathways associated with melanogenesis (Lin and Hu, 2021).

Molecular Docking

Molecular docking is a computational method used to predict the binding affinity and interaction modes between ligands and receptors, allowing the assessment of antihyperpigmentation potential through the analysis of hydrogen bonds, hydrophobic interactions, and binding to active site residues (Seeliger and De Groot, 2010). The receptor used in this study was the tyrosinase enzyme (PDB: 2Y9X), downloaded from the RCSB Protein Data Bank and prepared using AutoDock Tools by removing water molecules, adding polar hydrogens, calculating Gasteiger charges, and saving the structure in PDBQT format (Sadeghian *et al.*, 2024).The bioactive compounds identified from the network pharmacology analysis were obtained from PubChem in SDF format and converted to PDB using PyMOL, followed by further preparation in AutoDock Tools through the addition of polar hydrogens, charge assignment, and definition of rotatable

bonds (Seeliger and De Groot, 2010). Docking was performed using AutoDockTools with a grid box centered on the active site of tyrosinase at coordinates x = 10.044, y = 28.706, z = 43.443 and dimensions of 14 × 14 × 14 Å, enabling exploration of ligand orientations within the catalytic pocket (Mechqoq *et al.*, 2022). Docking protocol validation was performed by redocking the native ligand into the active site of tyrosinase to evaluate the reliability of the docking parameters. The validation produced an RMSD value of 1.19 Å, indicating that the docking protocol was valid and reliable because RMSD values below 2.0 Å are generally considered acceptable for reproducing experimental binding poses. The pose with the lowest binding affinity was selected as the most stable conformation (Nazir *et al.*, 2022), and interactions were analyzed using Discovery Studio Visualizer (DSV) to identify relevant interacting residues and molecular orientations involved in tyrosinase inhibition (Kausar *et al.*, 2024).

Result and Discussion

Network Pharmacology

a. Bioactive Collection by Literature Review

Through a search using the keyword "Persimmon Vinegar Compounds" on Google Scholar, various bioactive compounds in persimmon vinegar were successfully identified based on reviews from several scientific publications. Component selection was carried out by referring to the listed literature and through a comparative literature analysis, in which compounds with high dominance and accuracy levels were selected. Google Scholar played a role in filtering studies that met eligibility criteria and provided sufficient methodological information. In addition, searches using the same keyword were also conducted on other major scientific databases, such as Web of Science and ScienceDirect, to expand the scope and validity of the search results. The following is the result of the bioactive compound collection from persimmon vinegar.

Table 1. Bioactive Compounds in Persimmon Vinegar

No	Secondary metabolite properties	molecular group	PubChem CID	References
1.	catechin	flavonoids	73160	(Bayram, Ozkan and Sagdic, 2020), (Yaqub *et al.*, 2016), (Kim *et al.*, 2020)
2.	epicatechin	flavonoids	72276	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
3	epigallocatechin	tannin	72277	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
4	ferulic acid	Hydroxycinnamic acids	445858	(Athanasiadis *et al.*, 2023), (Hosseininejad *et al.*, 2022)
5	p-coumaric acid	Hydroxycinnamic acids	637542	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
6	gallic acid	hydroxybenzoic acids	370	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)

7	α-carotene	carotenoids	6419725	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
8	β-carotene	carotenoids	5280489	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
9	β-cryptoxanthin	carotenoids	21421799	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
10	proanthocyanins	Flavonoids	32387820	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
11	zeaxanthin	carotenoids	5280899	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
12	catechol	benzenediol	289	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
13	caffeic acid	Hydroxycinnamic acids	689043	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
14	quinic acid	hydroxybenzoic acids	37439	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
15	rutin	flavonoids	5280805	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
16	hesperidin	flavonoids	10621	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
17	quercetin	Flavonoids	5280343	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
18	protocatechuic acid	flavonoids	72	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
19	syringic acids	hydroxybenzoic acid	10742	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
20	malic acid	dicarboxylic acid and the hydroxy acid	525	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
21	aspartic acid	non-essential amino acid	5960	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
22	citrulline	non-essential amino acid	9750	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
23	GABA	essential amino acid	119	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
24	tryptophan	essential amino acid	6305	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
25	phenylalanine	essential aromatic amino acid	6140	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)

26	citric acid	tricarboxylic acid	311	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)
27	kaempferol	flavonoids	5280863	(Yaqub *et al.*, 2016), (Bayram, Ozkan and Sagdic, 2020), (Hosseininejad *et al.*, 2022), (Kim *et al.*, 2020)

b. Target Proteins Collection by GeneCards

GeneCards provides target proteins and proteins associated with hyperpigmentation. It is the world's largest human-centric database, offering integrated genetic, genomic, and biological information to help researchers understand human health and disease. This comprehensive and searchable database contains easily accessible details about every known and predicted human gene, making GeneCards an integrative resource. The following are the results of the target gene screening for persimmon vinegar.

Table 2. Target Proteins Screening Results

Compound Type	Number of Gene Targets	Gifts Range	Relevance Range
Gallic Acid	58	55-33	47.9-10.01
P-Coumaric Acid	32	61-50	42.06-10.41
Ferulic Acid	29	61-56	41.97-10.05
Protocatechuic acid	3	66-19	42.43-10.01
Quercetin	14	66-50	13.63
(Epi)catechin	1	19	11.07
Cathecol	5	21-64	10.04-86.45
Caffeic acid	46	21-69	10.04-143.01
Quinic acid	16	31-66	11.26-37.37
Syringic acids	37	31-67	10.1-42.24
Malic acid	48	7-68	10.02-120.62
Citrulline	18	54-63	10.01-43.34
Tryptophan	29	4-63	10.13-64.14
Phenylalanine	11	9-63	10.37-107.12
Citric acid	51	25-69	10-107.12
Aspartic acid	79	1-69	10.01-142.95

Based on Table 2, using persimmon vinegar-associated target proteins with a relevance score ≥10.00, a total of 477 protein targets were identified, accumulated from 10 bioactive compound components. Among these 477 compounds, several molecular targets were found to be identical. After further screening, 386 heterogeneous molecular targets were obtained, as illustrated in the Venn diagram (Figure 1).

c. Disease-Related Target Gene Collection and Network by DisGeNET Cytoscape App

Based on the screening results of the 386 persimmon vinegar-related target proteins, further filtering was performed to identify target proteins associated with hyperpigmentation using DisGeNET. The following results illustrate the relationship between persimmon vinegar target proteins and hyperpigmentation:

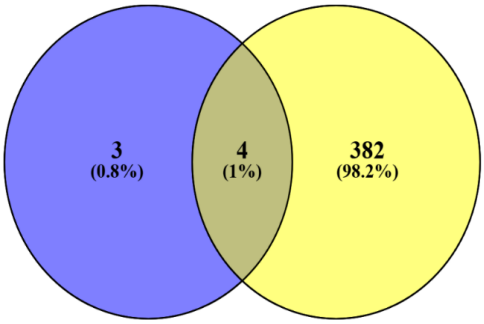


Figure 1. Venn diagram of target proteins: persimmon vinegar (yellow) and hyperpigmentation (blue)

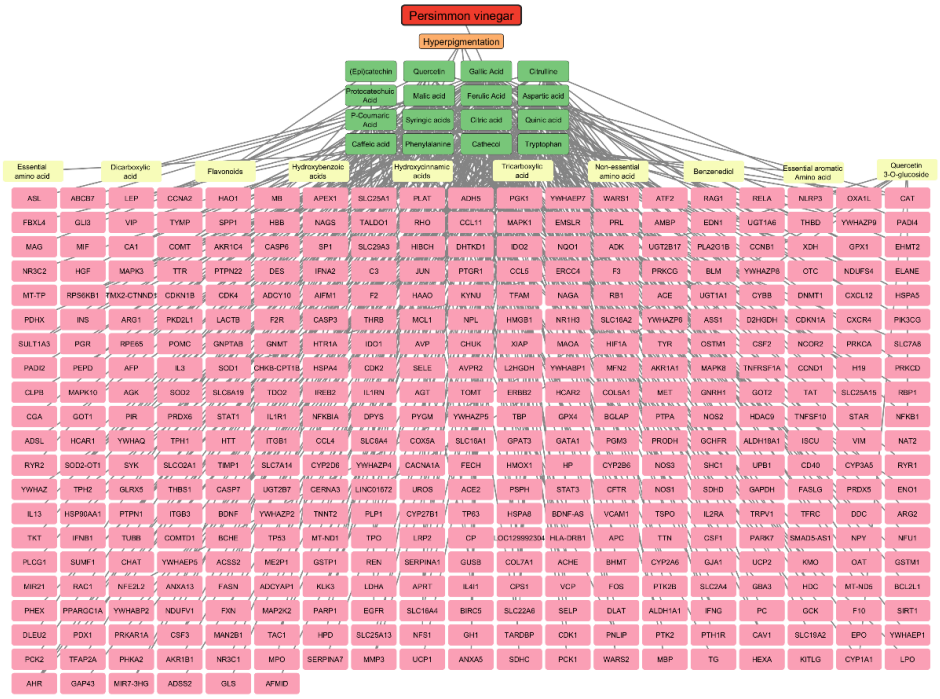


figure 2. Visual network of Persimmon vinegar (red: plant name, yellow: compound molecules, green: bioactive components, orange: disease, pink: target proteins)

Table 3. 4 Target Proteins of Persimmon Vinegar Related to Hyperpigmentation

Target Proteins	Compound Type	Score
SLC29A3 (Equilibrative nucleoside transporter 3)	Aspartic acid	13.89
AHR (Aryl hydrocarbon receptor)	Aspartic acid, Gallic acid	11.82
COL7A1 (Collagen alpha-1(VII))	Aspartic acid	10.75
MFN2 (Mitofusin-2)	Aspartic acid, Malic acid	10.04

Based on Figure 1, four out of seven hyperpigmentation-related target proteins (57.14%) overlapped with the target proteins associated with persimmon vinegar. In addition, these overlapping targets represented 1.04% of the total 386 persimmon vinegar-related target proteins identified. The overlapping proteins were associated with the compound groups aspartic acid, gallic acid, and malic acid, as presented in Table 3.

d. PPI network and Pathway enrichment Analysis

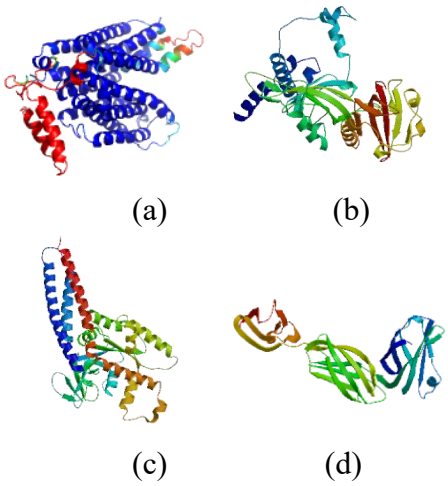


Figure 3. 4 core proteins interaction networks from the STRING database (PPI enrichment p-value: 1, 4 nodes) (a: SLC29A3, b: AHR, c: COL7A1, d: MFN2)

SLC29A3 is a member of the sodium or hydrogen exchanger (NHE) family that plays a key role in regulating ionic homeostasis within melanosomes, organelles responsible for melanin synthesis and storage in melanocytes. In the context of skin pigmentation, SLC29A3 is crucial in maintaining the melanosomal pH, a critical factor determining the activity level of enzymes involved in melanin synthesis, particularly tyrosinase. SLC29A3 functions by exchanging sodium ions (Na^+) from the cytoplasm with hydrogen ions (H^+) from the melanosome lumen. This process leads to the efflux of H^+ ions from the melanosome, directly contributing to an increase in melanosomal pH. A higher pH within melanosomes creates a more favorable environment for tyrosinase activity, the key enzyme in melanin biosynthesis. Without the correct pH, tyrosinase becomes inactive or degraded, thereby reducing melanin production. Therefore, the presence and activity of SLC29A3 are essential in determining the efficiency of the melanogenesis pathway (Snyman *et al.*, 2024).

Apart from SLC29A3, another target gene contributing to the molecular mechanisms of hyperpigmentation is AHR. Scientific discourse validates that the Aryl Hydrocarbon Receptor (AHR), a ligand-activated transcription factor, is stimulated by several ligands such as Benzo[a]pyrene (from cigarette smoke and vehicle emissions), FICZ (a tryptophan metabolite), and TCDD (an industrial contaminant). Activation of AHR increases the expression of melanogenesis enzymes (TYR, TYRP1, DCT) through interaction with XRE (xenobiotic response elements). Additionally, AHR amplifies the expression of melanogenesis genes via the MITF transcription pathway (Li *et al.*, 2024). In summary, AHR activity increases when stimulated by aromatic pollutant compounds like dioxins and PAHs, tryptophan metabolites, and UV-induced ligands, leading to the activation of MITF, the master transcription factor in melanogenesis.

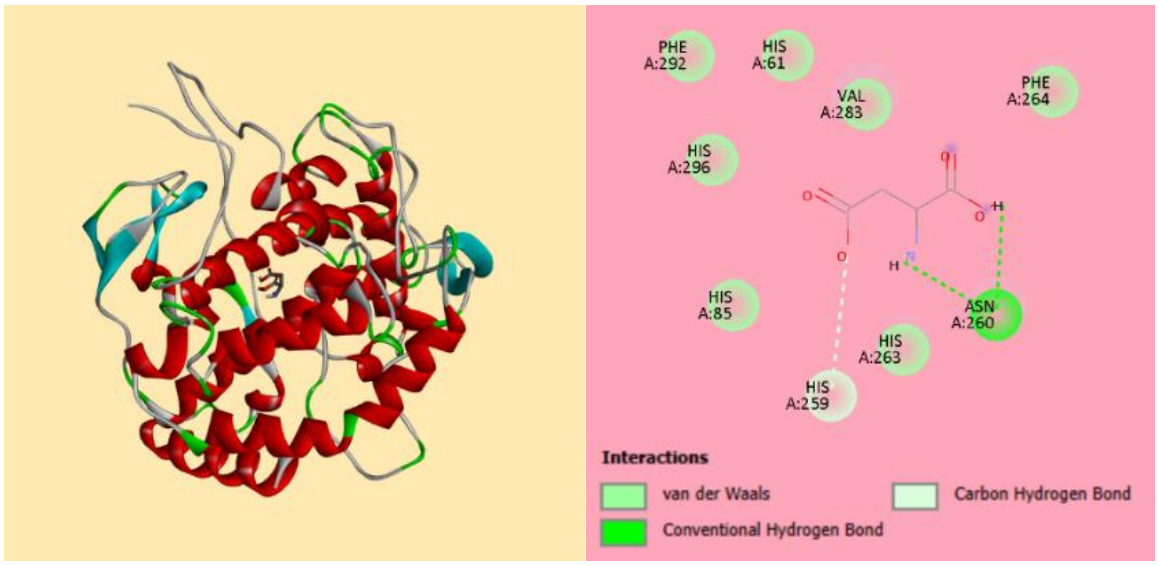
COL7A1 (Collagen Type VII Alpha 1 Chain) is a critical component of anchoring fibrils at the skin's basement membrane that stabilizes adhesion between epidermal cells (including keratinocytes and melanocytes) and the dermis. Beyond maintaining structural integrity, damage or mutations in collagen VII can disrupt melanocyte behavior such as excessive migration and decreased adhesion through indirect mechanisms, including activation of inflammatory pathways (NF-κB/TNF-α) and disturbance of ECM-integrin signaling. In conditions like burns or acne scars, damage to COL7A1 contributes to localized melanin

accumulation known as post-inflammatory hyperpigmentation (PIH). Meanwhile, COL7A1 mutations (e.g., in Dystrophic Epidermolysis Bullosa) cause melanin leakage into the dermis due to basement membrane disruption, making the skin appear darker (Markiewicz *et al.*, 2022). In other words, mutation or damage to COL7A1 is a cascade factor in hyperpigmentation.

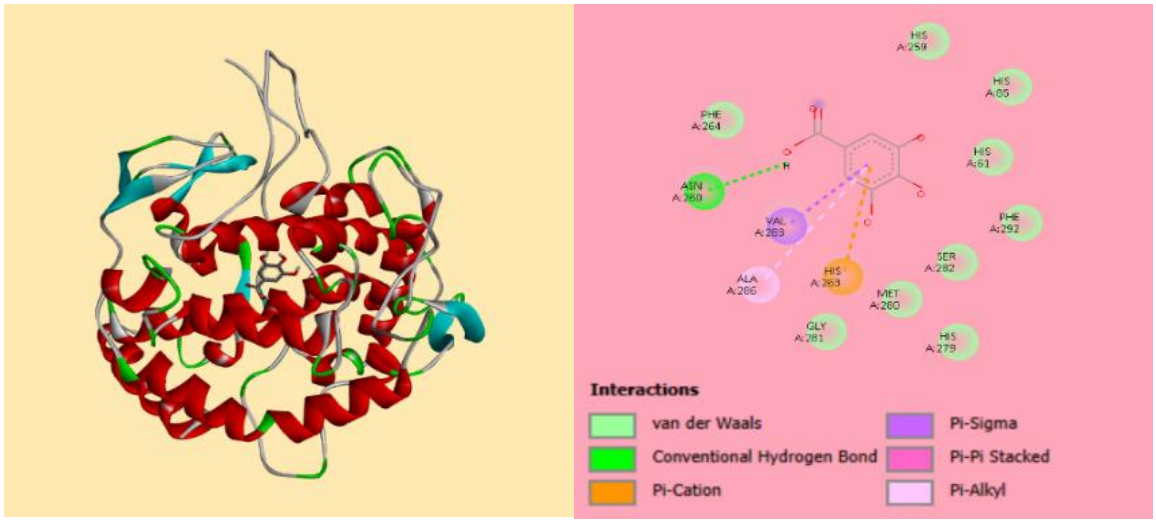
MFN2 (Mitofusin 2) deficiency is also associated with melasma or hyperpigmentation. MFN2 is a GTPase protein located on the outer mitochondrial membrane and is responsible for regulating the clearance of dysfunctional mitochondria through autophagy. If MFN2 is deficient, damaged mitochondria are not eliminated, leading to increased Reactive Oxygen Species (ROS). ROS accumulation induces intracellular oxidative stress, which triggers p38 phosphorylation, enhancing MITF activity and hindering melanosome autophagy by lysosomes. In relation to melanosome modulation, MFN2 acts as a degradation agent for damaged or excess melanosomes via a specialized autophagy pathway. Reduced MFN2 expression, whether transient or stable, activates melanosome retention mechanisms, leading to uncontrolled melanin production (Tanwar *et al.*, 2022).

Molecular Docking

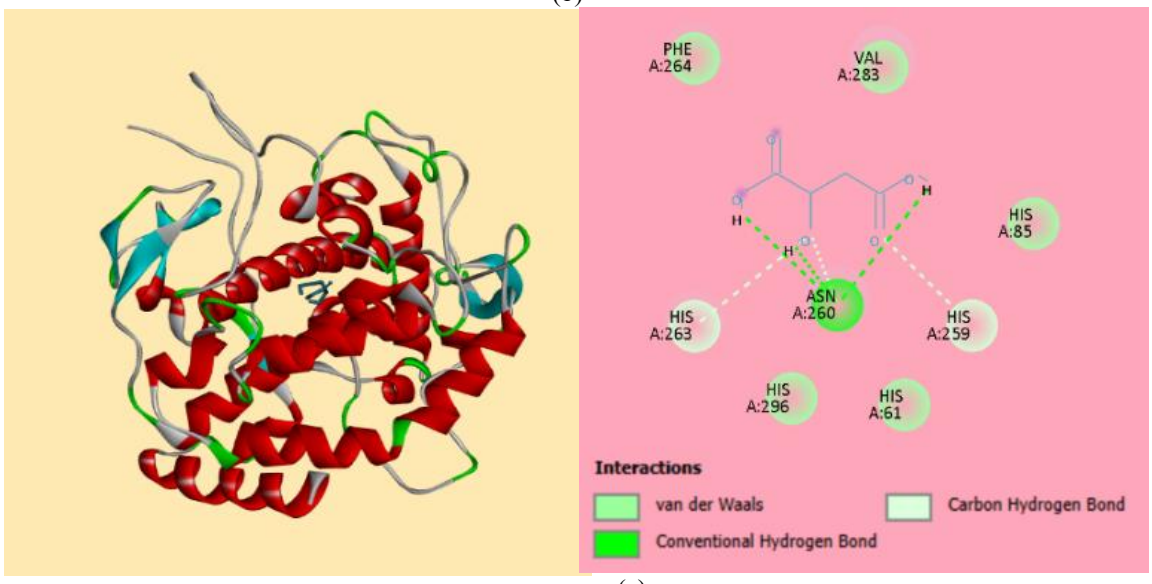
Molecular docking analysis was carried out to determine the binding affinity and interaction modes between the bioactive compounds of persimmon vinegar—namely aspartic acid, gallic acid, and malic acid and the melanogenesis-related target protein, tyrosinase (PDB: 2Y9X). These three compounds represent the organic acids and polyphenols identified through the network pharmacology approach. The docking visualizations are presented in Figure 4, illustrating the ligand orientations within the tyrosinase active pocket and the interacting amino acid residues (Zolghadri *et al.*, 2019).



(a)



(b)



(c)

Figure 4. Visualization of Molecular Docking Results of Persimmon Vinegar Bioactive Compounds against Tyrosinase (PDB: 2Y9X) (a: Aspartic acid, b: Gallic acid, c: Malic acid)

The docking results showed that aspartic acid interacts with polar residues located around the active site of tyrosinase. Aspartic acid forms two hydrogen bonds involving HIS259 and ASN260. HIS259 is one of the crucial residues surrounding the active pocket and has been frequently reported to stabilize ligands toward the Cu^{2+} catalytic center. The interaction between aspartic acid and HIS259 indicates that despite its simple and non-aromatic structure, the compound is able to occupy relevant regions of the active site through polar interactions. ASN260 is a polar residue that commonly functions as a hydrogen bond acceptor, making it highly compatible with hydrophilic compounds such as aspartic acid. These findings are consistent with reports that organic acids can bind to polar regions of tyrosinase even without forming π–π interactions (Ghasemi *et al.*, 2023).

Table 4. Binding Affinity Value and Interaction of Persimmon Vinegar Bioactive Compounds with Tyrosinase Enzyme (PDB: 2Y9X)

Ligand	Docking Score (kcal/mol)	Number Interaction	Residu Interacton (H-bond)
Aspartic acid	0.44	2	HIS259, ASN260
Gallic acid	-1.52	1	ASN260
Malic acid	0.51	2	HIS259, HIS263

Gallic acid exhibited the lowest binding energy among the tested persimmon vinegar compounds (−1.52 kcal/mol), indicating a relatively more stable interaction compared to aspartic acid and malic acid. However, its binding affinity remained weaker than that of the native ligand (−4.92 kcal/mol), suggesting that gallic acid may possess moderate inhibitory potential toward tyrosinase. Despite forming only one hydrogen bond with ASN260, the aromatic ring and phenolic hydroxyl groups of gallic acid may contribute through π–π interactions and hydrophobic contacts near the catalytic center, resulting in a stronger overall affinity than the other compounds. ASN260 is frequently reported as a key interacting residue for phenolic inhibitors due to its strategic position near the ligand entry pathway leading to the active site of tyrosinase (Zolghadri *et al.*, 2019). Therefore, despite the small number of hydrogen bonds, the stability of gallic acid binding arises from a combination of non-covalent forces rather than hydrogen bonding alone.

Malic acid formed two hydrogen bonds involving HIS259 and HIS263. HIS259 plays a crucial role due to its proximity to the Cu^{2+} catalytic center, whereas HIS263 helps stabilize ligand orientation toward the catalytic pocket. These interactions suggest that carboxylate-containing compounds such as malic acid have moderate potential in occupying positions that may influence tyrosinase activity, although their overall binding strength remains weaker than polyphenols like gallic acid. Previous studies report that histidine residues in tyrosinase (e.g., His85, His94, His259, His263) are key components of the Cu^{2+} cofactor system; therefore, ligands forming hydrogen bonds with these residues have a greater likelihood of modulating enzymatic activity (Ghasemi *et al.*, 2023). The presence of shared interacting residues (HIS259, ASN260) among the three ligands occurs because all compounds bind within the same active site of tyrosinase, which is highly specific to hyperpigmentation-related mechanisms, allowing them to act through similar inhibitory pathways (Mechqoq *et al.*, 2022).

Conclusion

Network identified 386 target proteins associated with the bioactive compounds of persimmon vinegar, with four proteins (SLC29A3, AHR, COL7A1, and MFN2) overlapping with hyperpigmentation-related targets. Molecular docking demonstrated that aspartic acid, gallic acid, and malic acid were able to interact with the active site of tyrosinase, with gallic

acid showing the most favorable binding affinity among the tested compounds. Overall, these findings suggest that persimmon vinegar possesses potential as a natural antihyperpigmentation agent and may serve as a promising candidate for further experimental and formulation studies.

Acknowledgement

We would like to thank the Ministry of Religion of the Republic of Indonesia through the Awakened Indonesia Research Funds program (MORA The AIR Funds Program) based on Decree Number 6841 of 2024 for the funding support that has enabled this research to be carried out well.

Funding Source

This research was funded by LPDP - Indonesia Endowment Fund for Education Agency in collaboration with the Ministry of Religion through the Awakened Indonesia Research Funds (MORA The AIR Funds Program) program with Decree Number 6841 of 2024.

References

Athanasiadis, V. *et al.* (2023) "Maximizing the Extraction of Bioactive Compounds from Diospyros kaki Peel through the Use of a Pulsed Electric Field and Ultrasound Extraction," *Biomass (Switzerland)*, 3(4), pp. 422–440. Available at: https://doi.org/10.3390/biomass3040025.

Bayram, Y., Ozkan, K. and Sagdic, O. (2020) "Bioactivity, Physicochemical and Antimicrobial Properties of Vinegar Made from Persimmon (*Diospyros kaki*) Peels," *Sigma Journal of Engineering and Natural Sciences*, 38, pp. 1643–1652.

Direito, R. *et al.* (2021) "From *Diospyros kaki* L. (persimmon) Phytochemical Profile and Health Impact to New Product Perspectives and Waste Valorization," *Nutrients*. MDPI. Available at: https://doi.org/10.3390/nu13093283.

Ghasemi, N. *et al.* (2023) "Thiazolopyrimidine Derivatives as Novel Class of Small Molecule Tyrosinase Inhibitor," *BMC Chemistry*, 17(1). Available at: https://doi.org/10.1186/s13065-023-01077-z.

Hosseininejad, S. *et al.* (2022) "Valorization of Persimmon Fruit through the Development of New Food Products," *Frontiers in Food Science and Technology*, 2. Available at: https://doi.org/10.3389/frfst.2022.914952.

Kausar, R. *et al.* (2024) "Synergistic Biomedical Potential and Molecular Docking Analyses of Coumarin–Triazole Hybrids as Tyrosinase Inhibitors: Design, Synthesis, In Vitro Profiling, and In Silico Studies," *Pharmaceuticals*, 17(4). Available at: https://doi.org/10.3390/ph17040532.

Kim, Y.M. *et al.* (2020) "Characterization of Bioactive Ligands with Antioxidant Properties of Kiwifruit and Persimmon Cultivars Using In Vitro and In Silico Studies," *Applied Sciences (Switzerland)*, 10(12), pp. 1–15. Available at: https://doi.org/10.3390/APP10124218.

Li, Y. *et al.* (2024) "The Role of Aryl Hydrocarbon Receptor In Vitiligo: A Review," *Frontiers in Immunology*. Frontiers Media SA. Available at: https://doi.org/10.3389/fimmu.2024.1291556.

Lin, Y. and Hu, Z. (2021) "Bioinformatics Analysis of Candidate Genes Involved in Ethanol-Induced Microtia Pathogenesis based on A Human Genome Database: GeneCards," *International Journal of Pediatric Otorhinolaryngology*, 142. Available at: https://doi.org/10.1016/j.ijporl.2020.110595.

Markiewicz, E. *et al.* (2022) "Post-Inflammatory Hyperpigmentation in Dark Skin: Molecular Mechanism and Skincare Implications," *Clinical, Cosmetic and Investigational Dermatology*. Dove Medical Press Ltd, pp. 2555–2565. Available at: https://doi.org/10.2147/CCID.S385162.

Matheus, J.R.V. *et al.* (2022) "Persimmon (*Diospyros kaki* L.): Chemical Properties, Bioactive Compounds and Potential Use in the Development of New Products–A Review," *Food Reviews International*. Taylor and Francis Ltd., pp. 384–401. Available at: https://doi.org/10.1080/87559129.2020.1733597.

Mechqoq, H. *et al.* (2022) "Molecular Docking, Tyrosinase, Collagenase, and Elastase Inhibition Activities of Argan By-Products," *Cosmetics*, 9(1). Available at: https://doi.org/10.3390/cosmetics9010024.

Moolla, S. and Miller-Monthrope, Y. (2022) "Dermatology: how to manage facial hyperpigmentation in skin of colour," *Drugs in Context*, 11, pp. 1–14. Available at: https://doi.org/10.7573/dic.2021-11-2.

Nautiyal, A. and Wairkar, S. (2021) "Management of hyperpigmentation: Current treatments and emerging therapies," *Pigment Cell and Melanoma Research*. John Wiley and Sons Inc, pp. 1000–1014. Available at: https://doi.org/10.1111/pcmr.12986.

Nazir, Y. *et al.* (2022) "Molecular Docking, Synthesis, and Tyrosinase Inhibition Activity of Acetophenone Amide: Potential Inhibitor of Melanogenesis," *BioMed Research International*, 2022. Available at: https://doi.org/10.1155/2022/1040693.

Noor, F. *et al.* (2022) "Network Pharmacology Approach for Medicinal Plants: Review and Assessment," *Pharmaceuticals*. MDPI. Available at: https://doi.org/10.3390/ph15050572.

Piñero, J. *et al.* (2021) "The DisGeNET Cytoscape App: Exploring and Visualizing Disease Genomics Data," *Computational and Structural Biotechnology Journal*, 19, pp. 2960–2967. Available at: https://doi.org/10.1016/j.csbj.2021.05.015.

Rathee, P. *et al.* (2021) "Skin Hyperpigmentation and its Treatment with Herbs: An Alternative Method," *Future Journal of Pharmaceutical Sciences*, 7(1). Available at: https://doi.org/10.1186/s43094-021-00284-6.

Rosyadah, M., Afendi, F.M. and Kusuma, W.A. (2017) "Penguraian Mekanisme Kerja Jamu dengan Menggunakan Analisis Graf Tripartit pada Jejaring Senyawa-Protein-Penyakit," *Jurnal Jamu Indonesia*, 2(1), 8-16.

Sadeghian, S. *et al.* (2024) "Synthesis, Biological Evaluation, Molecular Docking, MD Simulation and DFT Analysis of New 3-Hydroxypyridine-4-One Derivatives as Anti-Tyrosinase and Antioxidant Agents," *Heliyon*, 10(15). Available at: https://doi.org/10.1016/j.heliyon.2024.e35281.

Seeliger, D. and De Groot, B.L. (2010) "Ligand Docking and Binding Site Analysis with PyMOL and Autodock/Vina," *Journal of Computer-Aided Molecular Design*, 24(5), pp. 417–422. Available at: https://doi.org/10.1007/s10822-010-9352-6.

Snyman, M. *et al.* (2024) "The Metabolism of Melanin Synthesis—from Melanocytes to Melanoma," *Pigment Cell and Melanoma Research*. John Wiley and Sons Inc, pp. 438–452. Available at: https://doi.org/10.1111/pcmr.13165.

Stracke, C.M. and Trisolini, G. (2021) "A Systematic Literature Review on the Quality of Moocs," *Sustainability (Switzerland)*, 13(11). Available at: https://doi.org/10.3390/su13115817.

Tanwar, J. *et al.* (2022) "Mitofusin-2 Negatively Regulates Melanogenesis by Modulating Mitochondrial ROS Generation," *Cells*, 11(4). Available at: https://doi.org/10.3390/cells11040701.

Thawabteh, A.M. *et al.* (2023) "Skin Pigmentation Types, Causes and Treatment—A Review," *Molecules*. MDPI. Available at: https://doi.org/10.3390/molecules28124839.

Tjandrawinata, R.R. *et al.* (2022) "Mekanisme Molekuler Imunomodulasi oleh Huangqi (*Astragali radix*) menggunakan Sistem Farmakologi Jejaring," *Jurnal Ilmu Kefarmasian Indonesia*, 20(2). Available at: https://www.ncbi.nlm.nih.gov/gene/.

Xu, M. *et al.* (2019) "A Network Pharmacology Approach to Uncover the Molecular Mechanisms of Herbal Formula Kang-Bai-Ling for Treatment of Vitiligo," *Evidence-based Complementary and Alternative Medicine*, 2019. Available at: https://doi.org/10.1155/2019/3053458.

Yaqub, S. *et al.* (2016) "Chemistry and Functionality of Bioactive Compounds Present in Persimmon," *Journal of Chemistry*. Hindawi Limited. Available at: https://doi.org/10.1155/2016/3424025.

Zolghadri, S. *et al.* (2019) "A Comprehensive Review on Tyrosinase Inhibitors," *Journal of Enzyme Inhibition and Medicinal Chemistry*. Taylor and Francis Ltd, pp. 279–309. Available at: https://doi.org/10.1080/14756366.2018.1545767.