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In silico, in vitro and topical formulation of apigenin from *Apium graveolens* L. as a natural anti-hyperpigmentation agent

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Abstract. Apigenin from *Apium graveolens* L. was evaluated as a natural anti-hyperpigmentation agent using integrated *in silico*, *in vitro*, and topical formulation approaches. Molecular docking against melanogenesis related enzymes showed favorable binding energies toward tyrosinase (−7.5 kcal/mol), tyrosinase related protein 1 (TYRP1) (−7.9 kcal/mol), and dopachrome tautomerase (−7.2 kcal/mol). Antioxidant activity assessed by the thiobarbituric acid (TBA) assay demonstrated concentration-dependent inhibition of lipid peroxidation, reaching a maximum inhibition of 65.1% at 25 ppm ($p < 0.05$), followed by reduced activity at higher concentrations. Tyrosinase inhibitory assays revealed mild but significant concentration-dependent inhibition (11.1–38.2%) between 3,125 and 50,000 ppm. Apigenin was formulated into a carbomer 934 gel, exhibiting suitable viscosity and stable rheological properties over four weeks. These findings indicate that apigenin acts primarily as an antioxidant with supplementary tyrosinase modulation, supporting its potential use in stable topical anti-hyperpigmentation formulations.

1 Introduction

Skin hyperpigmentation has become a more frequently reported condition in recent years, representing a common dermatological issue encountered globally. A large-scale survey of 48,000 respondents across 34 countries found that nearly 50% of individuals worldwide experience at least one type of pigmentary disorder, including hyperpigmentation linked to ultraviolet (UV) exposure, aging, and chronic inflammation [1]. The global prevalence among adults is estimated at 15–25%. Hyperpigmentation results from an excessive accumulation of melanin, causing darker patches of skin. Although melanin protects against UV-induced damage, its overproduction is associated with uneven pigmentation and cosmetic concerns [2]. Excessive UV exposure triggers oxidative stress in skin cells, disrupts cellular homeostasis, damages DNA and proteins, and triggers inflammatory pathways. This

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environment promotes the expression of tyrosinase, a key enzyme in melanin synthesis, consequently enhancing melanogenesis [3].

Current therapeutic approaches primarily aim to attenuate oxidative stress and inhibit tyrosinase activity. Synthetic depigmenting agents, including hydroquinone and kojic acid, are widely employed in cosmetic formulations [4]. However, hydroquinone has been banned for cosmetic use in Indonesia by the National Agency of Drug and Food Control (BPOM No. 23 of 2019) due to adverse effects, including irritation, erythema, vitiligo, exogenous ochronosis, and carcinogenicity [5]. The use of kojic acid is limited to 1% concentration because higher doses may induce allergic reactions and dermatitis. These restrictions highlight the need for safer, more tolerable alternatives derived from natural sources.

Among promising natural compounds, apigenin, a flavonoi commonly found in celery *(Apium graveolens)*, parsley, and chamomile, has gained interest for its potential to modulate pigmentation [6]. Anti-hyperpigmentation agents generally work by inhibiting enzymes involved in melanin synthesis, including tyrosinase, D-dopachrome tautomerase, and tyrosinase-related protein 1 [7]. Apigenin's antioxidant capacity also contributes indirectly by scavenging ROS. Moreover, apigenin reduces lipid peroxidation and malondialdehyde (MDA) production, protecting cells from oxidative stress-induced damage [8].

Previous studies have investigated apigenin and celery extracts for cosmetic and pharmaceutical applications; however, most remain preliminary and lack comprehensive evaluation. A 2025 study demonstrated that celery extract exhibits high antioxidant activity in cosmetic formulations but did not specifically assess the effect of apigenin on tyrosinase inhibition [9]. Another study reported the potential of apigenin as a tyrosinase inhibitor using *in silico* approaches, yet lacked subsequent *in vitro* validation [10]. Furthermore, apigenin was confirmed to interact strongly with the Cu^{2+} active site of mushroom tyrosinase (*Agaricus bisporus*), although its efficacy in topical formulations has not yet been evaluated [11]. Based on these gaps, the present study was designed to integrate *in silico* analysis of tyrosinase with *in vitro* assays of antioxidant activity (TBA method, IC_{50}) and tyrosinase inhibition (TIA, IC_{50}) to support the development of a topical formulation containing apigenin derived from celery.

2 Methods and materials

2.1 General procedures

This study was conducted as an experimental laboratory investigation consisting of three sequential stages, namely *in silico* analysis, *in vitro* evaluation, and topical formulation. The *in silico* stage was designed to predict the molecular interactions of apigenin derived from *Apium graveolens* with key proteins involved in melanogenesis, including tyrosinase, tyrosinase-related protein 1, and D-dopachrome tautomerase. The outcomes of the molecular docking analysis were used as a preliminary reference to support subsequent *in vitro* assays.

The *in vitro* stage focused on evaluating the biological activity of apigenin through antioxidant and tyrosinase inhibition assays. Antioxidant activity was assessed using the thiobarbituric acid method by measuring the inhibition of malondialdehyde formation as an indicator of lipid peroxidation. Tyrosinase inhibitory activity was evaluated to determine the potential of apigenin as an anti-hyperpigmentation agent. The final stage of the study

involved the formulation of apigenin into a topical gel dosage form, followed by physical characterization and stability evaluation to assess its suitability for topical application.

2.2 In silico study

Molecular docking analysis was conducted using a MacBook Air M1 equipped with an Apple M1 processor and 8 GB of RAM. The three-dimensional structure of apigenin was obtained from the PubChem database (SDF) (https://pubchem.ncbi.nlm.nih.gov). The target protein structures were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org), including tyrosinase (PDB ID: 2Y9X), tyrosinase-related protein 1 (PDB ID: 5M8M), and D-dopachrome tautomerase (PDB ID: 1DPT). Ligand and protein preparation was performed using BIOVIA Discovery Studio.

Molecular docking simulations were subsequently carried out using PyRx (Python Prescription) Virtual Screening Tool version 0.8. The resulting ligand–protein complexes were subsequently visualized in three dimensions using PyMOL to analyze binding conformations and interaction patterns.

2.3 Antioxidant activity assay using TBA method (Kikuzaki and Nakatani (1993))

The assay was conducted using standard laboratory glassware, including volumetric flasks and Erlenmeyer flasks, along with a UV–Visible spectrophotometer (Genesys 10 UV, 190–1100 nm) set at a wavelength of 532 nm and a Hettich Universal centrifuge. Apigenin isolate with a purity of 98% was used as the test sample. 1,1,3,3-Tetramethoxypropane (TMP) was employed as a standard for malondialdehyde (MDA) calibration, while butylated hydroxytoluene served as a positive control. A TMP standard curve was prepared at concentrations of 1, 5, 8, 10, 13, 15, 18, and 20 μM. For each concentration, 1 mL of TMP solution was mixed with 2 mL of 20% trichloroacetic acid (TCA) and 2 mL of 1% thiobarbituric acid (TBA) prepared in 50% acetic acid. The reaction mixtures were heated at 100°C for 10 minutes, cooled to room temperature, and centrifuged at 3000 rpm for 15 minutes.

Apigenin solutions were prepared at concentrations of 5, 10, 25, 50, 100, and 200 ppm using dimethyl sulfoxide in distilled water. Each sample solution was incubated at 40°C prior to analysis to prevent compound degradation. Subsequently, 1 mL of each solution was mixed with 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetic acid, followed by heating at 100°C for 10 minutes to allow formation of the TBA–MDA complex. After cooling, the mixtures were centrifuged at 3000 rpm for 15 minutes, and the absorbance was measured at 532 nm. The IC_{50} values determined the inhibition activity of the test sample. A blank consisting of DMSO treated under identical conditions was used for background correction. All measurements were performed in duplicate, and antioxidant activity was expressed as percentage inhibition of MDA formation. The percentage of inhibition was calculated using the formula of:

$$\%\text{Inhibition} = \frac{\text{MDA control} - \text{MDA sample}}{\text{MDA control}} \times 100\% \qquad (1)$$

2.4 Tyrosinase inhibitory assay

Tyrosinase inhibitory activity was assessed using a commercial assay kit. Apigenin samples were prepared at concentrations of 3.125, 6.250, 12.500, 25.000, and 50.000 ppm. The

inhibitory activity was determined based on enzymatic reaction measurements, and all analyses were performed in triplicate. Absorbance was measured at 300 nm, and tyrosinase inhibitory activity was expressed as percentage inhibition calculated using the formula of:

$$\%\text{Inhibition} = \frac{(\text{Abs control} - \text{Abs blank}) - \text{Abs sample}}{(\text{Abs control} - \text{Abs blank})} \times 100\% \quad (2)$$

2.5 Topical gel formulation and physical stability evaluation

The preparation of apigenin topical gel was conducted according to the method described by Ansel HC (2005), with minor modifications. Carbomer 934 was used as the gelling agent. Initially, all materials required for gel preparation were prepared, including carbomer 934, triethanolamine, propylene glycol, and hot distilled water. A total of 1.2 g of carbomer 934 was dispersed in hot distilled water under continuous trituration using a mortar and pestle until a homogeneous dispersion was obtained. Subsequently, triethanolamine and propylene glycol were gradually added to the carbomer dispersion while stirring continuously until a clear and viscous gel base was formed.

Apigenin powder was then incorporated into the gel base at appropriate amounts to obtain six different formulations with final concentrations of 5, 10, 25, 50, 100, and 200 ppm. The resulting formulations were mixed until uniform gels were obtained. Physical stability testing was performed by storing the gel formulations at two different temperatures, namely 28°C (room temperature) and 50°C, for a period of four weeks. Organoleptic properties, including color, odor, and texture, were observed to detect any physical changes during the storage period. Viscosity measurements were conducted using a Brookfield viscometer at a spindle speed of 20 rpm. The viscosity was recorded once the pointer reached a stable reading. Measurements were performed at week 1 and week 4, and the obtained viscosity data were analyzed in relation to shear rate to evaluate the rheological behavior of the gel formulations.

2.6 Data analysis

The IC_{50} value was defined as the concentration of the sample required to inhibit 50% of biological activity, including lipid peroxidation in the TBA assay and enzymatic activity in the tyrosinase inhibitory assay (TIA). Antioxidant and enzyme inhibitory activities were classified based on IC_{50} values as very strong ($IC_{50} < 50$ ppm), strong ($50 < IC_{50} < 100$ ppm), moderate ($100 < IC_{50} < 150$ ppm), weak ($150 < IC_{50} < 200$ ppm), and very weak ($IC_{50} > 200$ ppm). The experimental data were evaluated for normality and homogeneity of variance prior to statistical analysis. When the data met the assumptions of normality and homogeneity, differences among groups were analyzed using one-way analysis of variance (ANOVA). Statistical significance was determined at a confidence level of 95%, with p-values less than 0.05 considered statistically significant. When the data did not meet the assumptions of normality and homogeneity, the Kruskal–Wallis test was applied.

3 Result and discussion

3.1 Molecular docking analysis

Molecular docking was performed to evaluate the interaction of apigenin with three key enzymes involved in melanogenesis, namely tyrosinase (PDB ID: 2Y9X), tyrosinase-related protein 1 (TYRP1; PDB ID: 5M8M), and dopachrome tautomerase (DCT; PDB ID: 1DPT). This *in silico* study was conducted as a preliminary assessment to support subsequent *in vitro*

investigations. In molecular docking analysis, more negative binding energy values indicate stronger predicted ligand and protein interactions. As presented in Table 1, apigenin showed a favorable binding affinity toward all investigated targets. Among them, apigenin exhibited the highest binding affinity toward TYRP1, with a binding energy of −7.9 kcal/mol, followed by tyrosinase (−7.5 kcal/mol) and DCT (−7.2 kcal/mol). The stronger predicted affinity toward TYRP1 suggests that apigenin is structurally well suited for the functional region of this enzyme.

Despite TYRP1 showing the most negative binding energy, the difference in binding affinity between TYRP1 and tyrosinase was relatively small (approximately 0.4–0.5 kcal/mol) and falls within the deviation range commonly associated with docking scoring functions. Therefore, this difference was interpreted cautiously and not considered sufficient to indicate a substantial difference in biological target preference based solely on *in silico* results. Interaction analysis further revealed that apigenin formed stabilizing interactions with amino acid residues located in the functional regions of each enzyme. In the tyrosinase and apigenin complex, apigenin interacted with key histidine residues associated with the catalytic site and copper coordination, which are essential for enzymatic activity. In the TYRP1 complex, apigenin forms interactions with residues that contribute to ligand stabilization within the active domain, which may account for the slightly higher binding affinity observed. In contrast, the interaction profile with DCT appeared less extensive, consistent with its comparatively weaker binding energy
(Table 1).

Table 1. Docking results between target protein and the test compound

Protein target	Ligand	Binding energy (kcal/mol)	Amino acid residue
Tyrosinase (PDB ID: 2Y9X)	Native ligand (tropolone)	-6.1	His263, Met280, Val283, Ala286
	Apigenin	-7.5	Val248, His263, Ser282, Val283, Ala286
Tyrosinase related protein 1 (PDB ID: 5M8M)	Native ligand (kojic acid)	-5.8	His192, His381, Gly388, Gly389, Gln390, Ser394
	Apigenin	-7.9	Arg374, His381, Leu382
D-dopachrome tautomerase (PDB ID: 1DPT)	Native ligand (N/A)	-	-
	Apigenin	-7.2	Pro1, Lys32, Arg36, Asn38, Ser63, Gly108, Lys109

Although TYRP1 exhibited the highest predicted binding affinity, biological relevance was prioritized in determining the target for *in vitro* evaluation. Tyrosinase functions as the rate-limiting enzyme in melanogenesis, catalyzing the initial steps of melanin synthesis, whereas TYRP1 and DCT act downstream in the processing of melanin intermediates [12]. Consequently, inhibition of tyrosinase is expected to reduce melanogenic activity upstream, indirectly affecting downstream enzymes. Based on this rationale, *in vitro* assays in this study were focused on tyrosinase inhibition.

As shown in Figure 1, molecular docking analysis demonstrated that apigenin binds favorably within the active site of tyrosinase (PDB ID: 2Y9X), exhibiting a binding energy

of −7.5 kcal/mol, which is lower than that of the native ligand (−6.1 kcal/mol). Visualization of both 2D and 3D interaction maps revealed that apigenin occupies a binding pocket overlapping with the native ligand, suggesting a potential competitive mode of inhibition.

Apigenin was predicted to interact with key residues such as His263 and Ser282, which are frequently reported as catalytically relevant residues involved in tyrosinase activity and copper coordination within the active site [8]. In addition, hydrophobic interactions with Val248, Val283, and Ala286 were observed, contributing to the stabilization of the ligand–enzyme complex. The combination of polar and hydrophobic interactions indicates a stable binding conformation of apigenin within the tyrosinase active region.

Importantly, interaction with His263 is also involved in native ligand binding, supporting the hypothesis that apigenin may interfere directly with substrate access and enzymatic turnover. These findings are consistent with previous studies, which report that flavonoids inhibit melanogenesis primarily through direct interaction with tyrosinase, the rate-limiting enzyme [8].

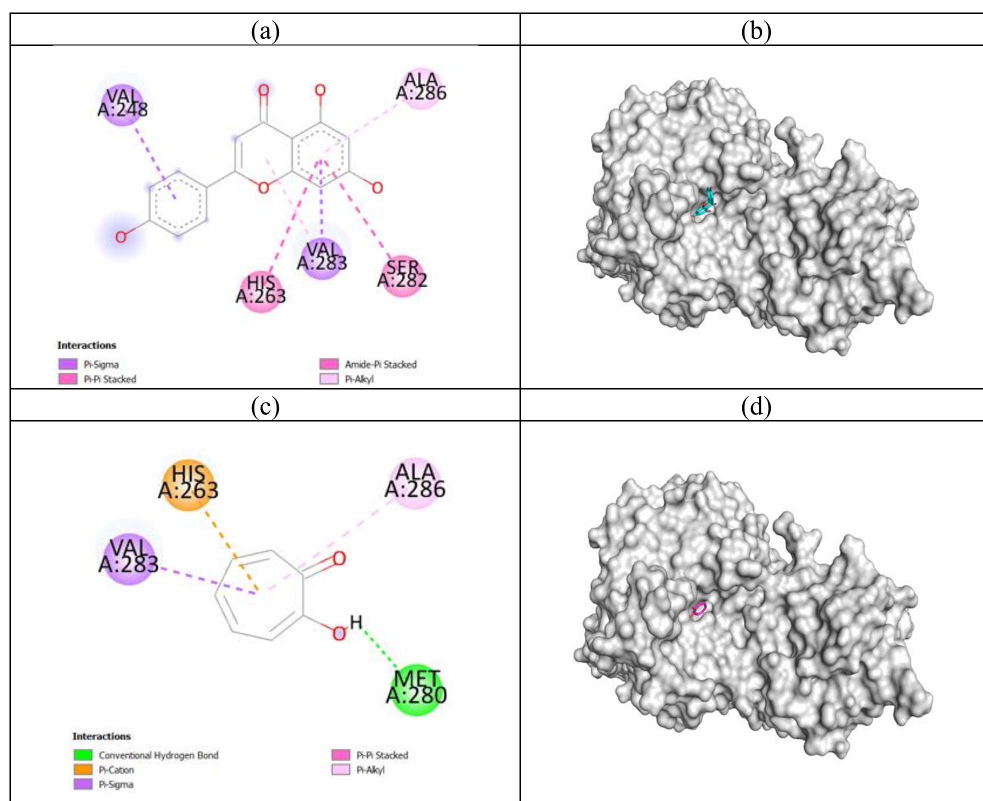

Fig. 1. In silico visualization bound to Tyrosinase (a) 2D visualization of Apigenin Bound to Tyrosinase (b) 3D visualization of Apigenin Bound to Tyrosinase (c) 2D visualization of Native Ligand Bound to Tyrosinase (d) 3D visualization of Native Ligand Bound to Tyrosinase.

The docking results for tyrosinase-related protein 1 (TYRP1; PDB ID: 5M8M), presented in Figure 2, showed that apigenin exhibited the strongest predicted binding affinity among the three targets, with a binding energy of −7.9 kcal/mol compared to −5.8 kcal/mol for the native ligand. Apigenin was predicted to interact with residues His381 and Arg374, which are involved in ligand stabilization within the TYRP1 binding pocket. His381 is a conserved residue and has been reported to play an important role in TYRP1–ligand interactions [4].

Although the binding affinity toward TYRP1 was slightly higher than that toward tyrosinase, the difference in binding energy (approximately 0.4–0.5 kcal/mol) is relatively small and likely falls within the intrinsic deviation range of molecular docking scoring functions. Moreover, from a biological standpoint, TYRP1 functions downstream of tyrosinase in the melanogenesis pathway and does not initiate melanin synthesis. Therefore, while the docking results suggest that apigenin can form a stable complex with TYRP1, this interaction is considered supportive rather than decisive for target prioritization. Similar observations have been reported for other flavonoids such as luteolin, which also show favorable docking toward TYRP1 but exert their primary depigmenting effects through tyrosinase inhibition [3].

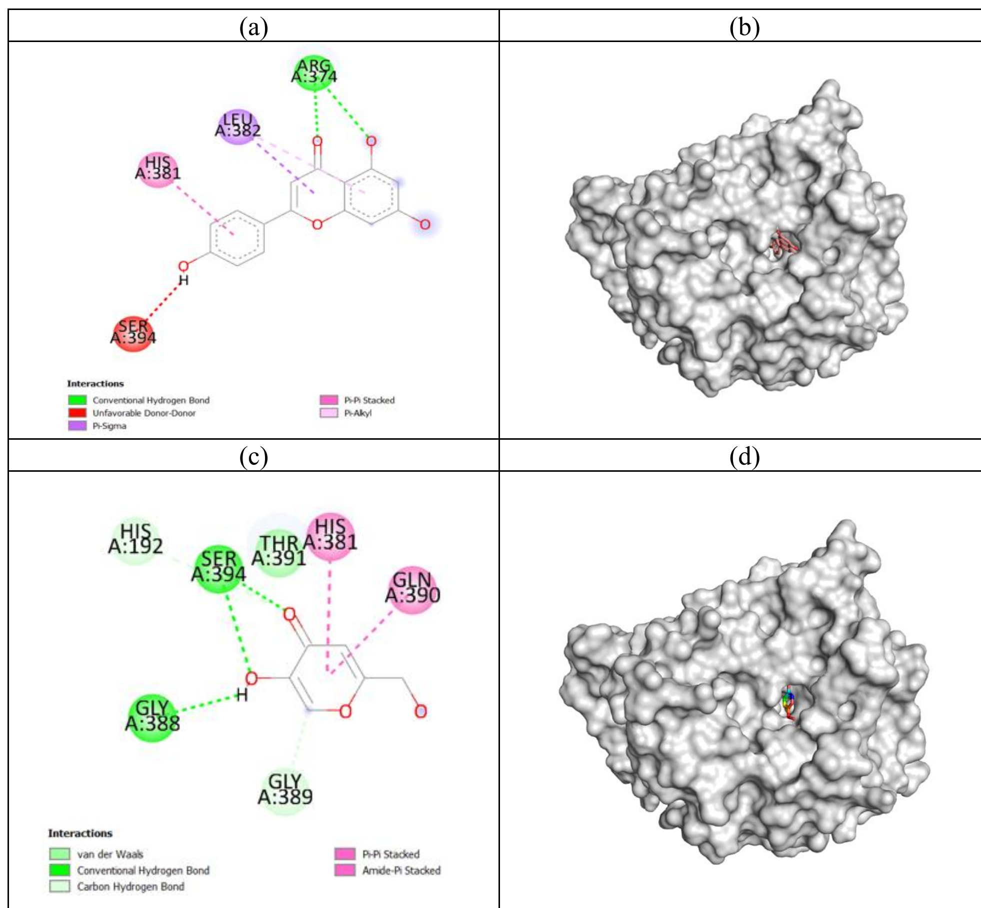

Fig. 2. In silico visualization bound to Tyrosinase related protein 1 (a) 2D visualization of Apigenin Bound to Tyrosinase related protein 1; (b) 3D visualization of Apigenin Bound to Tyrosinase related protein 1; (c) 2D visualization of Native Ligand Bound to Tyrosinase related protein 1; (d) 3D visualization of Native Ligand Bound to Tyrosinase related protein 1.

As illustrated in Figure 3, apigenin also demonstrated a stable binding conformation with dopachrome tautomerase (DCT; PDB ID: 1DPT), yielding a binding energy of −7.2 kcal/mol. Apigenin interacted with multiple polar and positively charged residues, including Lys32, Arg36, Asn38, Ser63, and Lys109, suggesting the formation of several potential hydrogen bonds and electrostatic interactions. Such interactions are known to contribute significantly to ligand stability within enzyme binding pockets [12].

Despite the favorable interaction profile, DCT acts at a later stage of melanogenesis by catalyzing the conversion of dopachrome into downstream melanin intermediates. Consequently, inhibition of DCT alone is less effective in reducing overall melanin synthesis compared to inhibition of tyrosinase [4]. Therefore, the interaction between apigenin and DCT is interpreted as complementary, potentially enhancing the overall anti-melanogenic effect rather than serving as the primary mechanism of action.

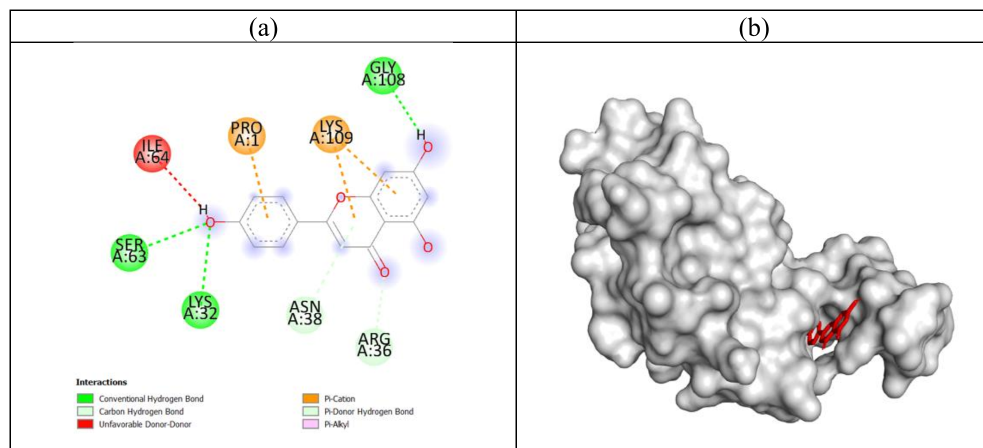

Fig. 3. In silico visualization bound to D-dopachrome tautomerase (a) 2D visualization of Apigenin Bound to D-dopachrome tautomerase; (b) 3D visualization of Apigenin Bound to D-dopachrome tautomerase.

Taken together, the molecular docking results indicate that apigenin exhibits multi-target binding potential toward key enzymes involved in melanogenesis. Although the strongest predicted affinity was observed with TYRP1, the small difference in binding energy relative to tyrosinase is not considered biologically decisive. Considering that tyrosinase is the rate-limiting enzyme in melanogenesis, while TYRP1 and DCT function downstream, tyrosinase remains the most relevant primary target for *in vitro* validation [8]. These findings provide a strong molecular rationale for subsequent experimental evaluation of apigenin using tyrosinase inhibition assays and support its potential application as a natural anti-hyperpigmentation agent.

3.2 Antioxidant activity of apigenin

The antioxidant activity of Apigenin was evaluated using the Thiobarbituric Acid (TBA) assay, which measures inhibition of lipid peroxidation via malondialdehyde (MDA) formation, a key marker of oxidative degradation of lipids. As shown in Figure 4, Apigenin exhibited a concentration-dependent inhibitory effect on lipid peroxidation, with percent inhibition increasing from 47.4% at 5 ppm to 55.9% at 10 ppm, reaching a maximum of 65.1% at 25 ppm.

Interestingly, higher concentrations resulted in a gradual decrease in activity, with 50 ppm showing 61.8%, 100 ppm 50.0%, and 200 ppm 30.9% inhibition. This bell-shaped response suggests that Apigenin has an optimal effective concentration range, while higher doses may reduce activity due to solubility limitations or potential molecular aggregation that diminishes interaction with peroxidized lipids [6, 11].

Statistical analysis of the data confirmed that differences in percent inhibition among the concentrations were significant, with p-values < 0.05. This indicates that the observed variations in antioxidant activity across concentrations are not due to random variation but reflect genuine concentration-dependent effects of Apigenin. The statistical significance

further strengthens the reliability of the data and supports the identification of 25 ppm as the optimal concentration for maximal lipid peroxidation inhibition.

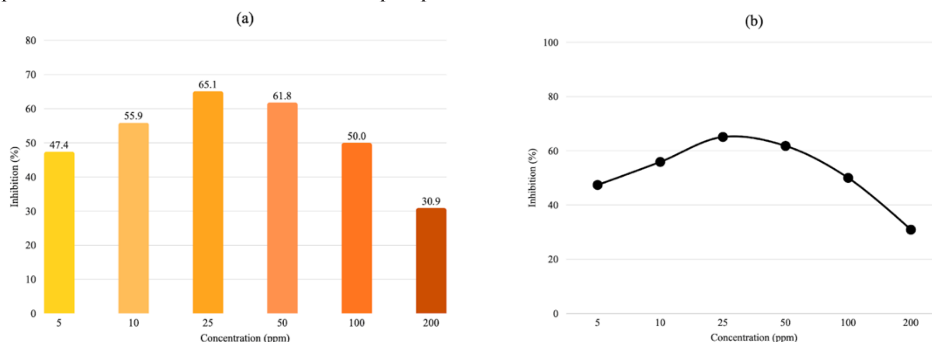

Fig. 4. Antioxidant activity of Apigenin (TBA assay) at various concentrations (a) Bar chart showing percent inhibition at each concentration (b) Line chart showing bell-shaped concentration-dependent inhibition with peak activity at intermediate concentrations.

The choice of the TBA assay over DPPH radical scavenging assay is particularly relevant for Apigenin, a semi-polar flavone. While DPPH is commonly employed to assess antioxidant activity, it is most effective for compounds that are either highly polar or non-polar and can freely donate electrons to the stable DPPH radical. Apigenin's semi-polar nature limits its solubility and interaction in DPPH-based assays, and preliminary tests demonstrated lower and inconsistent activity under this system. In contrast, the TBA assay evaluates inhibition of lipid peroxidation in a lipid-rich medium, which better reflects Apigenin's antioxidant potential in biological membranes and topical applications [2, 6].

The decline in inhibition at concentrations above 25 ppm may also relate to the chemical behavior of Apigenin in solution. High concentrations can lead to molecular aggregation or precipitation, reducing effective interaction with lipid radicals [8]. Similar trends have been observed in flavonoid formulations, including apigenin gel, where intermediate concentrations demonstrated higher ex vivo antioxidant activity than higher doses [8]. The potent antioxidant effect of apigenin observed in this study is relevant to skin protection and anti-aging applications.

Oxidative stress-induced lipid peroxidation contributes to membrane damage, melanogenesis, and accelerated skin aging [1, 7]. By inhibiting lipid peroxidation effectively, Apigenin may prevent ROS-mediated cellular damage and hyperpigmentation, supporting its potential inclusion in topical anti-aging formulations. Moreover, previous studies have reported that Apigenin modulates MAPK and NF-κB signaling pathways in keratinocytes and melanocytes, further contributing to its protective and anti-melanogenic effects [3,6]. From a formulation perspective, the identification of 25 ppm as the optimal concentration ensures maximal antioxidant efficacy while maintaining compliance with regulatory limits for cosmetic ingredients [5]. Higher concentrations do not improve activity and may introduce solubility or stability issues. These results underscore the importance of selecting appropriate assay systems, such as the TBA assay, for semi-polar flavonoids, rather than relying solely on radical-based methods like DPPH [2, 8].

3.3 Tyrosinase inhibition assay

The inhibitory effect of Apigenin on tyrosinase activity was assessed using the method described by Arguelles (2021), which employs a four-parameter logistic (4PL) sigmoidal fit, with concentration as the independent variable [4]. As shown in Figure 5, Apigenin began to exhibit measurable inhibition starting at 3,125 ppm, indicating that lower concentrations were

insufficient to interact effectively with the enzyme. Percent inhibition increased progressively with concentration 11.13% at 3,125 ppm, 14.67% at 6,250 ppm, 21.07% at 12,500 ppm, 33.09% at 25,000 ppm, and 38.23% at 50,000 ppm. Statistical analysis confirmed the significance of these differences ($p < 0.05$), demonstrating that the concentration-dependent increase is reliable and not due to experimental variability.

Despite the high concentrations tested, maximal inhibition did not reach 50%, and therefore the IC_{50} could not be reliably estimated, which classifies Apigenin as a relatively weak tyrosinase inhibitor under these in vitro conditions [4, 7, 10]. This mild inhibitory capacity may reflect the semi-polar nature of Apigenin, which affects its solubility and interaction with the enzyme's active site. Furthermore, enzyme saturation appears to occur near 50,000 ppm, as evidenced by the minimal increase in inhibition between 25,000 and 50,000 ppm, indicating a plateau phase where additional concentration increases no longer significantly enhance activity.

The linear trend observed in tyrosinase inhibition contrasts markedly with the bell-shaped response seen in the TBA antioxidant assay, where maximal inhibition of lipid peroxidation occurred at intermediate concentrations (25–50 ppm) and decreased at higher doses [6, 8]. This difference can be explained by the distinct underlying mechanisms of the two assays. In the TBA assay, antioxidant activity depends on the interaction between Apigenin molecules and lipid radicals in a semi-polar medium. High concentrations can lead to aggregation or precipitation, reducing the effective molecular surface area and thereby decreasing activity. In contrast, tyrosinase inhibition is determined by direct binding to the enzyme's active site, a process that remains largely proportional to concentration until the enzyme approaches saturation. Hence, the TIA data exhibit an approximately linear increase in inhibition within the tested concentration range [2, 10].

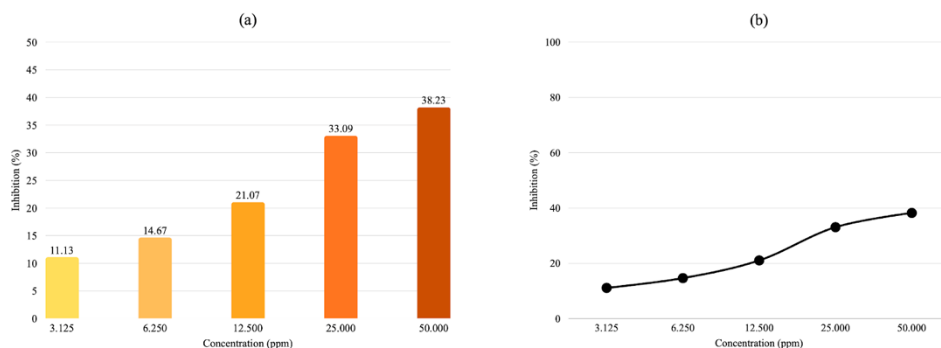

Fig. 5. Tyrosinase inhibitory activity of apigenin at various concentrations (a) Bar chart showing percent inhibition at each concentration (b) Line chart showing concentration-dependent inhibition and plateau at high concentration

The dual pattern bell-shaped antioxidant activity vs linear tyrosinase inhibition highlights the multifaceted bioactivity of Apigenin. While moderate concentrations optimize lipid peroxidation inhibition (TBA), substantially higher concentrations are necessary to achieve observable enzyme inhibition (TIA). This discrepancy underscores the importance of choosing appropriate assays based on the chemical nature of the compound and the biological target. In this case, TBA effectively captures Apigenin's semi-polar antioxidant behavior, whereas TIA directly assesses enzymatic modulation in a highly concentrated context [2, 6]. Mechanistically, Apigenin likely interacts with tyrosinase via hydrogen bonding and π-π stacking at the active site, thereby reducing the conversion of L-DOPA to melanin [4, 7].

Although the inhibition is modest, the effect is concentration-dependent, and the plateau at 50,000 ppm suggests that the binding sites of the enzyme are approaching saturation. This behavior aligns with molecular docking studies showing limited binding energy for Apigenin

compared to more potent tyrosinase inhibitors [10]. From a cosmetic and skin-protective perspective, these results indicate that Apigenin has potential as a mild anti-melanogenic agent, which, in combination with its potent antioxidant activity observed in the TBA assay, may offer dual protective effects against oxidative stress and hyperpigmentation [1, 3]. Formulation strategies could enhance solubility, increase effective concentration at the skin interface, and potentially improve tyrosinase inhibition in topical applications [8].

In conclusion, Apigenin exhibits statistically significant, concentration-dependent tyrosinase inhibition starting from 3,125 ppm and plateauing at 50,000 ppm ($p < 0.05$). Although IC_{50} was not reached within the tested range, these findings support the compound's potential as a multifunctional bioactive agent, capable of mitigating oxidative stress and modulating melanogenesis, with implications for anti-aging and skin-whitening formulations. The linear trend of TIA inhibition, in contrast to the bell-shaped TBA response, underscores the importance of assay selection in evaluating the activity of semipolar flavonoids like Apigenin [4, 11].

3.4 Topical gel formulation and physical stability evaluation

The viscosity profile of the apigenin carbomer gels showed a consistent increase as the concentration of apigenin rose from 5 to 200 ppm (Fig. 6). This behavior is characteristic of carbomer based gel systems and is primarily attributed to an increase in the effective solid content within the polymer matrix. The presence of additional dispersed apigenin molecules restricts polymer chain mobility and enhances resistance to flow. Upon proper neutralization, carbomer forms an expanded three dimensional network capable of incorporating active compounds without structural disruption. Therefore, the observed increase in viscosity represents normal rheological behavior rather than an indication of formulation instability [12].

From a formulation perspective, the gradual increase in viscosity with increasing apigenin concentration is advantageous for topical application. Gels with insufficient viscosity may exhibit poor retention on the skin surface and lead to uneven distribution of the active compound. In contrast, excessively viscous formulations can negatively affect spreadability and patient acceptability. In this study, all formulations exhibited viscosity values within a range considered suitable for topical gels, indicating an appropriate balance between mechanical strength and ease of application. This suggests that incorporation of apigenin up to 200 ppm does not compromise the functional performance of the gel system [13].

The comparison of viscosity values between week one and week four provides further insight into the physical stability of the formulations during storage. A slight increase in viscosity over time was observed across all concentrations, which may be associated with continued polymer hydration or gradual rearrangement of the carbomer network. Importantly, no decrease in viscosity was detected, indicating the absence of polymer degradation or phase separation. The relatively stable viscosity profile over the four week storage period demonstrates that the gel structure remains intact and resistant to environmental stress under the tested conditions [14].

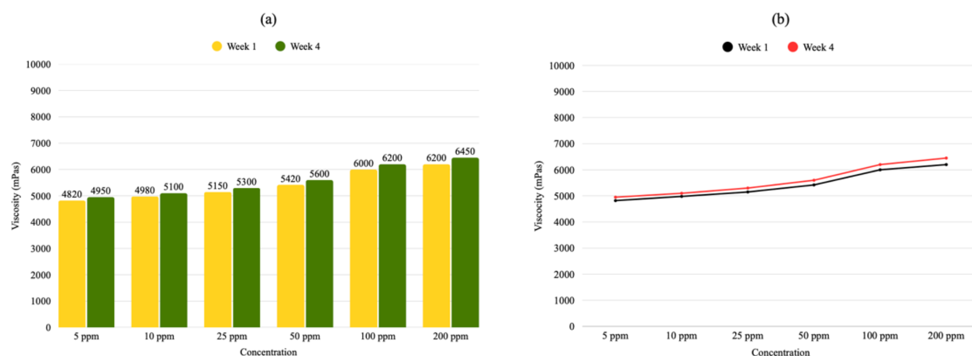

Fig. 6. Viscosity profile of apigenin carbomer gel formulations at different concentrations and storage times (a) bar chart bar chart showing viscosity values of gel formulations at each concentration (b) line chart line chart illustrating the concentration dependent trend of viscosity.

Overall, the stability of viscosity over time confirms the robustness of the carbomer 934 gel matrix in accommodating apigenin. Stable rheological behavior indicates that interactions between apigenin and the polymer network do not adversely affect gel integrity. Similar trends have been reported in recent studies on carbomer hydrogels, where increased loading of dispersed compounds resulted in higher viscosity while maintaining storage stability when formulation conditions were optimized [15]. These findings support the suitability of carbomer 934 as a reliable carrier for apigenin in topical anti hyperpigmentation formulations.

4 Conclusions

Apigenin from *Apium graveolens* shows consistent interactions with key enzymes involved in melanogenesis based on an integrated *in silico*, *in vitro*, and topical formulation approach. Molecular docking analysis demonstrated that apigenin binds favorably to tyrosinase, tyrosinase-related protein 1, and D-dopachrome tautomerase, with binding energies comparable to or lower than those of the native ligands. Although the strongest predicted affinity was observed toward tyrosinase-related protein 1, tyrosinase remains the most biologically relevant primary target due to its role as the rate-limiting enzyme in melanin synthesis. The interaction profiles indicate that apigenin is stabilized within the active regions of these enzymes through a combination of polar and hydrophobic interactions, providing a molecular rationale for its potential anti-hyperpigmentation activity.

In vitro assays showed that apigenin exhibits notable antioxidant activity, as indicated by effective inhibition of lipid peroxidation in the TBA assay, with optimal activity observed at intermediate concentrations. Tyrosinase inhibition was concentration-dependent but relatively mild, suggesting that apigenin acts as a modulatory rather than a strong direct inhibitor of the enzyme. Incorporation of apigenin into a carbomer-based gel resulted in physically stable formulations with suitable viscosity and no significant changes during storage. Overall, these findings support apigenin as a multifunctional natural compound with potential application in topical anti-hyperpigmentation formulations, while emphasizing the need for further formulation optimization and advanced biological evaluation.

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