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A Comparative Study on the Biological Activities of a Synthetic Peptide (Acetyl Hexapeptide-3) and a Natural Bee Product (Apilarnil): Antioxidant Capacity, Enzyme Inhibitory Activities, and Cytotoxicity

Sibel DIKMEN KUCUK*[1], Meral KEKECOGLU[2]

[1]*Düzce University, Coordination Office of Specialization in Environment and Health Technologies, 81620, Düzce*
[2]*Düzce University, Faculty of Arts and Sciences, Department of Biology, 81620, Düzce*

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Abstract

Skin aging involves the progressive dysregulation of extracellular matrix-degrading enzymes and oxidative stress pathways, driving demand for biologically characterized cosmeceutical ingredients. This study provides the first direct comparative evaluation of apilarnil , a lyophilized drone larvae-derived bee product (*Apis mellifera*) and acetyl hexapeptide-3 (Argireline®; Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2), a synthetic neurotransmitter inhibitor peptide, with respect to total antioxidant capacity, elastase, collagenase, and human tyrosinase inhibitory activities, and cytotoxicity toward HaCaT human keratinocytes. Compositional characterization of apilarnil revealed a protein-rich profile (42.67 g/100 g) containing 16 amino acids, multiple vitamins, and mineral constituents. Acetyl hexapeptide-3 exhibited higher total antioxidant capacity (5304.7 ± 103.9 µM Trolox equivalents) compared to apilarnil (645.3 ± 23.3 µM TE), attributable to the abundance of electron-donating residues in its defined sequence. Acetyl hexapeptide-3 also produced potent, concentration-dependent inhibition of elastase (IC_{50} = 1.534 mg/mL) and collagenase (IC_{50} = 0.00811 mg/mL), whereas apilarnil displayed a biphasic hormetic dose–response for both enzymes, with low-dose activation followed by high-dose inhibition. Neither compound showed an apparent inhibitory effect on human tyrosinase in a single preliminary experiment (n = 1); given the lack of statistical replication, this finding should be considered exploratory and requires confirmatory replication. Cytotoxicity assessment demonstrated that apilarnil was non-toxic across all tested concentrations (0.3125–20 mg/mL; viability >90%), while acetyl hexapeptide-3 exhibited concentration-dependent cytotoxicity (CC_{50} = 5.34% v/v). These findings reveal complementary bioactivity profiles between the two compounds, suggesting potential value in further investigating their combined use in cosmeceutical formulations.

Keywords: Apilarnil, Acetyl hexapeptide-3, Apitherapy, Enzyme inhibition, Antioxidant capacity, Cytotoxicity.

1. INTRODUCTION

Skin aging is a complex, multifactorial biological process driven by the interplay of intrinsic factors including chronological aging, genetic predisposition, and hormonal changes , and extrinsic factors such as ultraviolet (UV) radiation, environmental pollutants, and lifestyle-related stressors (Hussein et al., 2025; Krutmann et al., 2021). At the molecular level, wrinkle formation, loss of firmness, and irregular pigmentation are visible manifestations of aging that are primarily attributable to the progressive degradation of extracellular matrix (ECM) components and the dysregulation of key enzymatic pathways (Martic et al., 2022; Makrantonaki & Zouboulis, 2007). Reactive oxygen species (ROS) generated by UV exposure activate matrix metalloproteinases (MMPs) and serine proteases, accelerating the breakdown of collagen and elastin fibers that confer the skin's structural integrity and elasticity (Amano, 2009; Poljšak & Dahmane, 2012). In parallel, overactivation of tyrosinase, the rate-limiting copper-containing metalloenzyme in the melanogenesis pathway, catalyzes the hydroxylation of L-tyrosine to L-DOPA and its subsequent oxidation to dopaquinone, driving excessive melanin accumulation and contributing to hyperpigmentation disorders including melasma, solar lentigo, and freckles (Pillaiyar et al., 2017; Zolghadri et al., 2019).

*Sibel DIKMEN KUCUK; sibelkucuk@duzce.edu.tr

Given the central roles of these enzymatic pathways in skin aging, the inhibition of elastase, collagenase, and tyrosinase, alongside the maintenance of antioxidant homeostasis, has become a principal strategy in the development of cosmeceutical agents (Cruz et al., 2023; Jiratchayamaethasakul et al., 2020). In vitro enzyme inhibition assays targeting these enzymes, combined with cytotoxicity profiling on human keratinocyte cell lines, represent the most widely adopted and reproducible approach for the preliminary evaluation of cosmeceutical candidates (Cruz et al., 2023). The global cosmeceutical market continues to expand rapidly, driven by consumer demand for efficacious yet biologically safe active ingredients derived from both natural and synthetic sources (Crous et al., 2024).

Among synthetic cosmeceutical peptides, acetyl hexapeptide-3 (Argireline®; Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2; MW: 888.99 g/mol) represents one of the most extensively studied and commercially utilized anti-aging compounds. Acetyl hexapeptide-3, widely referred to as a "botox-like" peptide, has received considerable attention for its potential to reduce wrinkles dynamically through the modulation of neuromuscular activity and is used in topical formulations intended for anti-aging effects, scar treatment, and skin rejuvenation. Its mechanism of action involves competitive binding to the N-terminal domain of SNAP-25, destabilizing the SNARE complex and thereby inhibiting acetylcholine exocytosis and reducing repetitive contraction of the intrinsic muscles of facial expression (Blanes-Mira et al., 2002; Khvotchev & Soloviev, 2022). Clinical investigations have demonstrated wrinkle depth reductions of up to 30% following 30 days of twice-daily treatment with formulations containing 10% Argireline (Wang et al., 2013). Based on their mechanism of action, cosmeceutical peptides can be classified into signal peptides, carrier peptides, neurotransmitter inhibitor peptides, and enzyme inhibitor peptides; acetyl hexapeptide-3 belongs to the neurotransmitter inhibitor category and is considered an alternative option for botulinic products.

In parallel, honeybee-derived products used in apitherapy have attracted growing scientific interest as sources of multifunctional bioactive compounds with cosmeceutical potential. Apitherapy is a type of biotherapy that uses bees and their products as medicinal or preventative measures, promoting healing through reducing inflammation, enhancing circulation, and inducing healthy immunological responses; the field now encompasses natural factors related to beehives, including apilarnil. Apilarnil, composed of 3–7-day-old drone larvae (*Apis mellifera*), is a rich biological matrix containing proteins, free amino acids, lipids, vitamins, mineral salts, and bioactive hormones including testosterone and progesterone (Yücel et al., 2019; El-Wahed et al., 2024). Apilarnil is an organic bee product known to be rich in protein; phytochemical analysis by LC/MS/MS has identified numerous bioactive compounds, with prominent constituents including quinic acid, fumaric acid, aconitic acid, kaempferol, and quercetin. Previous studies have demonstrated its antioxidant activity, neuroprotective properties, hepatoprotective effects, and enzyme inhibitory activity against carbonic anhydrase isoenzymes and cholinesterases (Inci et al., 2023; Elashal et al., 2024). Apilarnil functions as an antioxidant, provides neuroprotection, boosts fertility, and possesses antiviral capabilities; these properties are supported by its diverse chemical composition comprising mineral salts, vitamins, carbohydrates, lipids, hormones, and amino acids. Despite this growing body of evidence, apilarnil has not been systematically compared with established synthetic peptides in terms of dermocosmetic potential, particularly inhibitory activity against enzymes associated with skin aging.

To the best of our knowledge, no study has previously conducted a direct comparative evaluation of apilarnil and acetyl hexapeptide-3 with respect to elastase, collagenase, and tyrosinase inhibitory activities, total antioxidant capacity, and cytotoxicity on human keratinocytes. The present study aimed to address this gap by providing a comprehensive side-by-side biological characterization of these two structurally and compositionally distinct compounds, thereby contributing to the evidence base for their potential application in cosmeceutical formulations.

2. MATERIAL AND METHOD

2.1. Materials

Lyophilized apilarnil, derived from 7-day-old drone larvae (*Apis mellifera*), was used as the bee-derived test compound. Acetyl hexapeptide-3 (Argireline®; Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2; MW: 888.99 g/mol; CAS: 616204-22-9; purity ≥98% by HPLC) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Porcine pancreatic elastase, bacterial collagenase (Type IA), human tyrosinase, and all associated assay substrates and

buffers were of analytical grade and sourced from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were of analytical or cell culture grade and procured from certified commercial suppliers.

2.2. Production and lyophilization of apilarnil

Apilarnil was produced under controlled apicultural conditions. Drone brood production was stimulated by introducing pre-fabricated drone comb foundations into selected hives, which simultaneously served as a biological control measure against *Varroa destructor* infestation. On day 7 post-oviposition, drone larvae were aseptically harvested from comb cells using sterile instruments, rinsed with distilled water to remove surface debris, and transferred to sterile glass containers (Sidor & Dzugan, 2020). The collected material was homogenized with a tissue homogenizer, and the resulting homogenate was lyophilized (LABFREEZ FD-10F-RE) at −55 °C for 72 h. Lyophilized APL was stored at −20 °C until use.

2.3. Compositional characterization of test compounds

Amino acid composition of lyophilized apilarnil was determined following acid hydrolysis of protein fractions, with subsequent analysis by ultrafast liquid chromatography coupled with ultraviolet detection (UFLC-UV); individual amino acids were quantified against validated reference standards (Jensen et al., 2019). Proximate composition — including moisture, ash, protein, fat, carbohydrate, and dietary fiber — was determined by standard analytical methods; lipid content was assessed following acid hydrolysis, and energy value was calculated using Atwater conversion factors expressed as kcal/100 g dry weight (Asoro et al., 2017). Vitamins were quantified by HPLC using fluorescence detection (HPLC-FLD) for water- and fat-soluble vitamins and ultraviolet detection (HPLC-UV) for vitamin D_3, with external calibration against certified standards (Zachara et al., 2017). Calcium and magnesium contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following acid digestion and expressed as mg/kg dry weight.

Acetyl hexapeptide-3 was obtained as a commercial lyophilized powder (Sigma-Aldrich, St. Louis, MO, USA). Theoretical amino acid composition was calculated from the known peptide sequence by stoichiometric analysis. Physicochemical properties including molecular formula, molecular weight, and sequence identity were confirmed by the manufacturer's certificate of analysis and are consistent with published reference data (Gorouhi & Maibach, 2009).

2.4. Antioxidant capacity assays

Total antioxidant capacity (TAC) of both test compounds was determined using the Antioxidant Assay Kit (MAK334-1KT, Merck, Darmstadt, Germany), based on the reduction of Cu^{2+} to Cu^{+} by antioxidant species in the sample, with subsequent colorimetric detection of the Cu^{+}–dye complex at 570 nm. Trolox was used as the calibration standard, and results were expressed as μM Trolox equivalents (μM TE). All measurements were performed in triplicate.

2.5. Enzyme inhibition assays

Inhibitory activities of both test compounds against elastase, collagenase, and tyrosinase were evaluated using concentration-dependent assay formats. Serial dilutions were prepared for each compound, and residual enzyme activities were expressed as percentages relative to untreated controls (defined as 100%).

Elastase inhibitory activity was measured using a fluorescence-based kit (EnzChek™ Elastase Assay Kit, E12056, Invitrogen, Waltham, MA, USA). Hydrolytic cleavage of a fluorophore–quencher-labeled peptide substrate by elastase generates a fluorescent signal proportional to enzyme activity. Test compounds at increasing concentrations were incubated with the enzyme–substrate mixture, and fluorescence was recorded at 505/513 nm (excitation/emission).

Collagenase inhibitory activity was assessed using a DQ-gelatin-based fluorescent kit (EnzChek™ Gelatinase/Collagenase Assay Kit, E12055, Invitrogen). Proteolytic cleavage of the heavily quenched fluorescein-conjugated substrate yields fluorescent products detectable at 495/515 nm. Compounds were co-incubated with collagenase and substrate at varying concentrations, and inhibition was calculated relative to enzyme-only controls. For both elastase and collagenase assays, compound-only controls (test compound and

substrate without enzyme) were included at each tested concentration to account for potential matrix interference from apilarnil, including intrinsic fluorescence, turbidity, or quenching effects; the corresponding compound-only signal was subtracted from the enzyme-containing reaction at each matching concentration prior to calculating residual enzyme activity.

Tyrosinase inhibitory activity was assessed using human tyrosinase derived from MNT-1 melanoma cell lysates. Cells were detached with 0.05% trypsin–EDTA, washed with PBS, and lysed in PBS containing 1% Triton X-100; lysates were clarified by centrifugation. Test compounds were prepared in DMSO and added to reaction mixtures at a final DMSO concentration of 5% (v/v). Reactions were initiated by adding L-DOPA (4 mM) and incubated at 37 °C for 4 h. Melanin formation was quantified at 600 nm, and inhibitory activity was expressed as percentage inhibition relative to DMSO-treated controls (Roulier et al., 2023). To confirm assay validity, reference positive-control inhibitors were included for each enzyme: N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone for elastase, 1,10-phenanthroline for collagenase, and kojic acid for tyrosinase.

Elastase, collagenase, and cytotoxicity assays were conducted in triplicate. The tyrosinase assay was performed as a single preliminary experiment (n = 1) due to the limited availability of MNT-1 cell lysate; results are presented as exploratory data.

2.6. Cell culture and cytotoxicity

HaCaT human keratinocyte cells were seeded at 5×10^3 cells/well in 96-well plates and allowed to adhere for 24 h at 37 °C under 5% CO_2. Cells were then exposed to serial dilutions of each test compound for 48 h. Viability was assessed using the AlamarBlue® assay (Invitrogen, Waltham, MA, USA), with fluorescence recorded at 560/590 nm (excitation/emission). Results were expressed as percentage viability relative to untreated controls, and CC_{50} values were determined from the resulting dose–response curves. All experiments were performed in triplicate. For acetyl hexapeptide-3, cytotoxicity was assessed using serial dilutions of the aqueous stock solution prepared for testing, and results are expressed relative to the undiluted stock (% v/v). As the exact concentration of this stock solution was not centrally recorded, cytotoxicity values could not be converted to an absolute concentration (mg/mL or μM) directly comparable to the units used in the enzyme inhibition assays; this is acknowledged as a limitation of the present study, and future work will report cytotoxicity data in absolute units to enable direct cross-assay comparison.

2.7. Statistical analysis

Data are presented as the mean ± SD of three technical replicates (n = 3), defined as repeated measurements conducted under identical conditions using the same sample preparation, except for the tyrosinase assay, which was performed as a single observation (n = 1). For elastase and collagenase assays, dose–response curves were fitted using a four-parameter sigmoidal (nonlinear regression) model, and IC_{50} values were derived from the resulting fits. For apilarnil, the biphasic dose–response profile, characterized by enzyme activation at low concentrations and inhibition at higher concentrations, was accounted for in curve fitting. CC_{50} values for cytotoxicity were similarly determined by nonlinear regression. Between-group differences in total antioxidant capacity were evaluated by Welch's unpaired t-test; statistical significance was set at $\alpha = 0.05$. All curve fitting and statistical analyses were performed using Python (scipy and matplotlib libraries). Results are expressed as percentage residual enzyme activity or cell viability relative to untreated controls. As the total antioxidant capacity data were derived from technical replicates of a single sample preparation rather than independent biological replicates, the resulting p-value and effect size should be interpreted as descriptive of this specific dataset rather than as generalizable inferential evidence.

3. RESULTS

3.1. Compositional characterization of test compounds

The theoretical amino acid composition of acetyl hexapeptide-3 and the experimentally determined amino acid composition of apilarnil are presented in Table 1 and Table 2, respectively.

Table 1. Theoretical amino acid composition of acetyl hexapeptide-3 (Argireline®)

Amino Acid Composition	Amount (mg/100 g)	Physicochemical Properties	Value
L-Arginine (Arg)	35,137	Molecular formula	$C_{34}H_{60}N_{14}O_{12}S$
L-Glutamic acid (Glu)	29,046	Molecular weight (g/mol)	888.99
L-Methionine (Met)	14,757	CAS number	616204-22-9
L-Glutamine (Gln)	14,413	Amino acid sequence	Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2
		Protein (g/100 g)	~100
		Carbohydrate (g/100 g)	0
		Fat (g/100 g)	0
		Moisture (g/100 g)	<5
		Purity (HPLC, %)	≥98

Table 2. Amino acid composition and proximate nutritional properties of apilarnil

Amino Acid Composition	Amount (mg/100 g)	Proximate Composition	Amount
L-Glutamic acid (Glu)	4718	Energy (kcal/100 g)	421
L-Lysine (Lys)	4675	Moisture (g/100 g)	6.12
L-Proline (Pro)	3121	Ash (g/100 g)	3.27
L-Aspartic acid (Asp)	3598	Protein (g/100 g)	42.67
L-Leucine (Leu)	2713	Carbohydrate (g/100 g)	34.85
L-Valine (Val)	2126	Dietary fiber (g/100 g)	3.55
L-Arginine (Arg)	1996	Fat (g/100 g)	9.96
L-Histidine (His)	1811	Vitamin E (mg/100 g)	0.32
L-Isoleucine (Ile)	1798	Vitamin B1 (mg/100 g)	0.11
L-Serine (Ser)	1783	Vitamin B3 (mg/100 g)	69.17
L-Threonine (Thr)	1605	Vitamin B6 (mg/100 g)	0.93
L-Tyrosine (Tyr)	1632	Vitamin C (mg/100 g)	26.36
L-Phenylalanine (Phe)	1558	Vitamin D3 (mg/100 g)	8.78
Glycine (Gly)	1584	Calcium (mg/kg)	689.3
L-Alanine (Ala)	1512	Magnesium (mg/kg)	983.1
L-Methionine (Met)	816		

Apilarnil demonstrated a complex nutritional profile, with a protein content of 42.67 g/100 g and a total of 16 identified amino acids; the most abundant were L-glutamic acid (4718 mg/100 g), L-lysine (4675 mg/100 g),

L-aspartic acid (3598 mg/100 g), and L-proline (3121 mg/100 g). In contrast, acetyl hexapeptide-3 is a chemically defined synthetic hexapeptide (Ac-Glu-Glu-Met-Gln-Arg-Arg-NH_2; MW: 888.99 g/mol) composed of four distinct amino acid types. Theoretical stoichiometric calculations indicated L-arginine as the most abundant residue (35,137 mg/100 g), followed by L-glutamic acid (29,046 mg/100 g), L-methionine (14,757 mg/100 g), and L-glutamine (14,413 mg/100 g).

3.2. Total antioxidant capacity

Total antioxidant capacity (TAC) values of acetyl hexapeptide-3 and apilarnil are presented in Figure 1. Acetyl hexapeptide-3 exhibited higher antioxidant capacity (5304.7 ± 103.9 µM Trolox equivalent) compared to apilarnil (645.3 ± 23.3 µM Trolox equivalent) (t = 75.79, df = 2.20, p = 0.000084, mean difference = 4659.4 µM TE, 95% CI [4416.71, 4902.09], Cohen's d = 61.88). As this comparison is based on technical replicates from a single sample preparation, this result should be interpreted as descriptive rather than confirmatory.

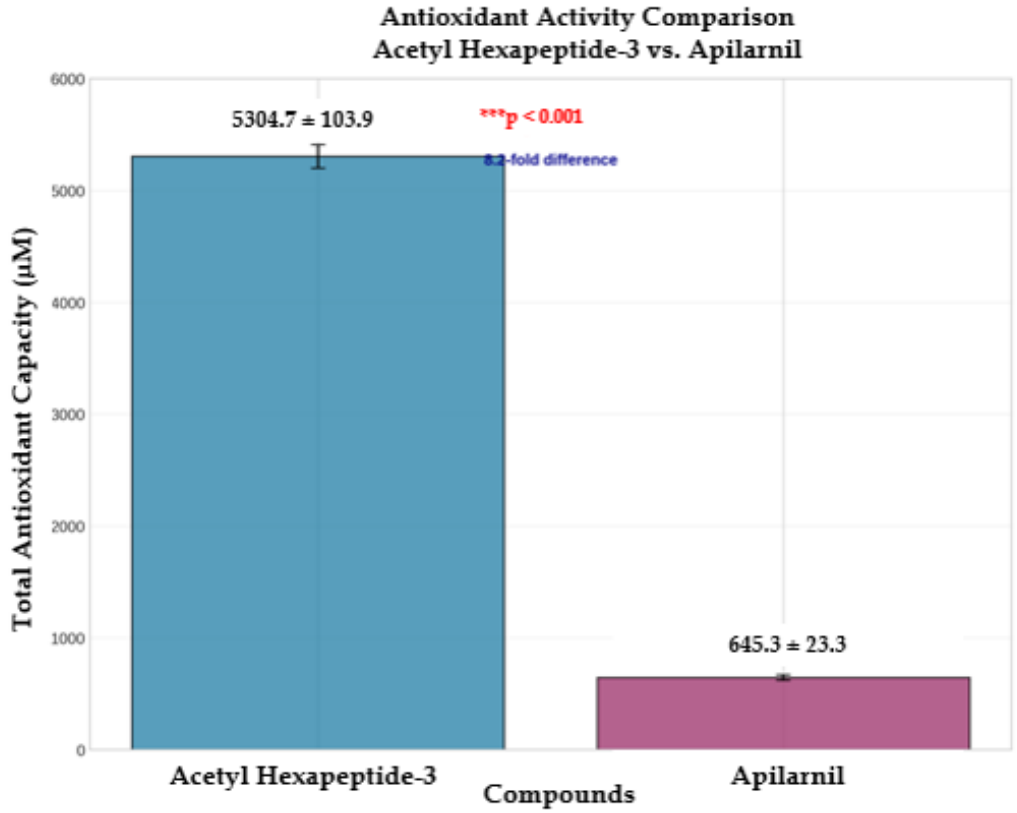

Figure 1. Total antioxidant capacity (TAC) of acetyl hexapeptide-3 and apilarnil

3.3. Elastase inhibitory activity

The effects of both compounds on porcine pancreatic elastase activity are illustrated in Figure 2. Acetyl hexapeptide-3 produced a classical concentration-dependent inhibition curve, with an IC_{50} value of 1.534 mg/mL. Apilarnil displayed a biphasic dose–response profile: at low concentrations (0.3125–2.5 mg/mL), residual enzyme activity exceeded 100%, indicating an apparent activation effect, followed by progressive inhibition at higher concentrations, with an extrapolated IC_{50} exceeding 20 mg/mL. The reference elastase inhibitor (N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone) confirmed assay validity, with an IC_{50} of 1.535% (vol: vol).

3.4. Collagenase inhibitory activity

Both compounds inhibited bacterial collagenase (Type IA) activity in a concentration-dependent manner, as shown in Figure 3. Acetyl hexapeptide-3 demonstrated potent inhibition with an IC_{50} of 0.00811 mg/mL. Apilarnil again exhibited a biphasic response, with activation at lower concentrations and inhibition at higher concentrations, yielding an IC_{50} of approximately 3.48 mg/mL. The reference collagenase inhibitor (1,10-phenanthroline) confirmed assay validity, with an IC_{50} of 0.0445 mM.

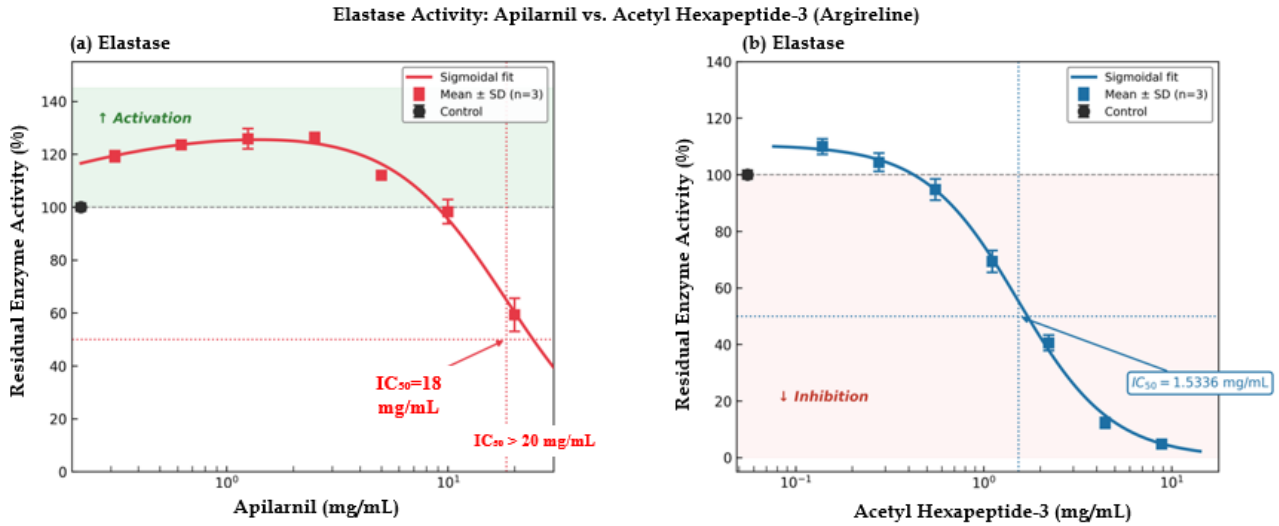

Figure 2. Inhibitory effects of apilarnil and acetyl hexapeptide-3 on porcine pancreatic elastase activity; (a) Apilarnil exhibited a biphasic dose–response profile with activation at low concentrations and inhibition at higher concentrations (extrapolated; IC_{50} > 20 mg/mL). (b) Acetyl hexapeptide-3 produced a classical sigmoidal inhibition curve (IC_{50} = 1.534 mg/mL). Data are presented as mean ± SD (n=3). Residual enzyme activity is expressed as a percentage of the untreated control

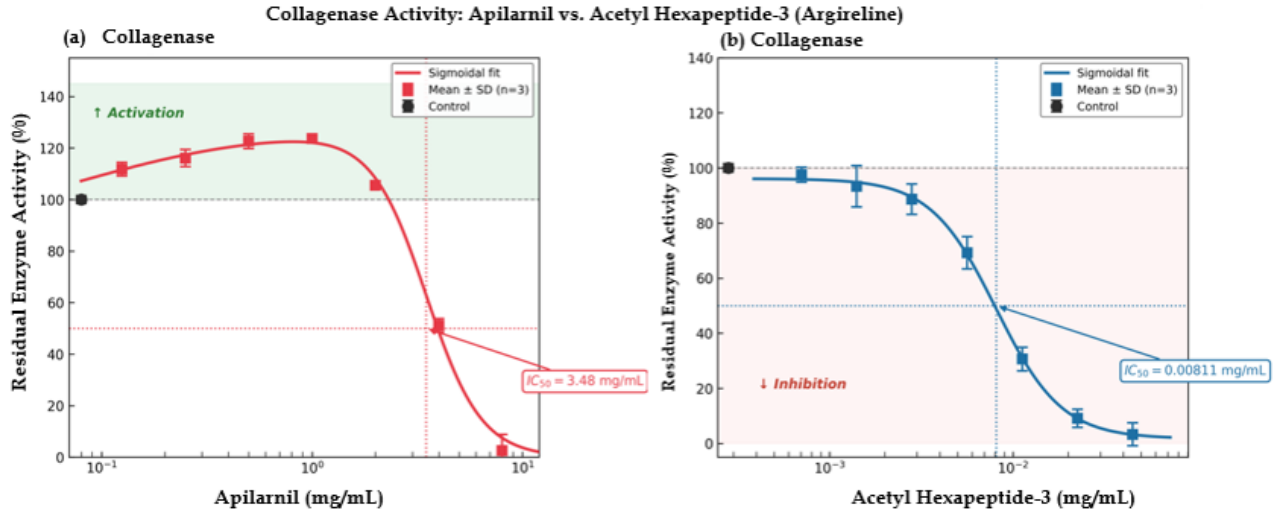

Figure 3. Inhibitory effects of apilarnil and acetyl hexapeptide-3 on bacterial collagenase (Type IA) activity; (a) Apilarnil exhibited a biphasic dose-response profile with activation at low concentrations and inhibition at higher concentrations (IC_{50} = 3.48 mg/mL). (b) Acetyl hexapeptide-3 produced a classical sigmoidal inhibition curve (IC_{50} = 0.00811 mg/mL). Data are presented as mean ± SD (n=3). Residual enzyme activity is expressed as a percentage of the untreated control

3.5. Tyrosinase inhibitory activity

In a preliminary single experiment (n=1), neither apilarnil nor acetyl hexapeptide-3 demonstrated meaningful inhibitory activity against human tyrosinase within the tested concentration ranges (Figure 4). Residual enzyme activity remained within 89.9–114.5% and 94.2–114.5% of control values for apilarnil and acetyl hexapeptide-3, respectively, with no discernible dose–response relationship observed for either compound. Accordingly, IC_{50} values could not be determined under the tested conditions. These results are considered exploratory and require confirmation with replicated experiments ($n \geq 3$). The reference tyrosinase inhibitor kojic acid confirmed assay validity, with an IC_{50} of 0.321 mM, demonstrating that the assay was capable of detecting genuine inhibitory activity under the tested conditions.

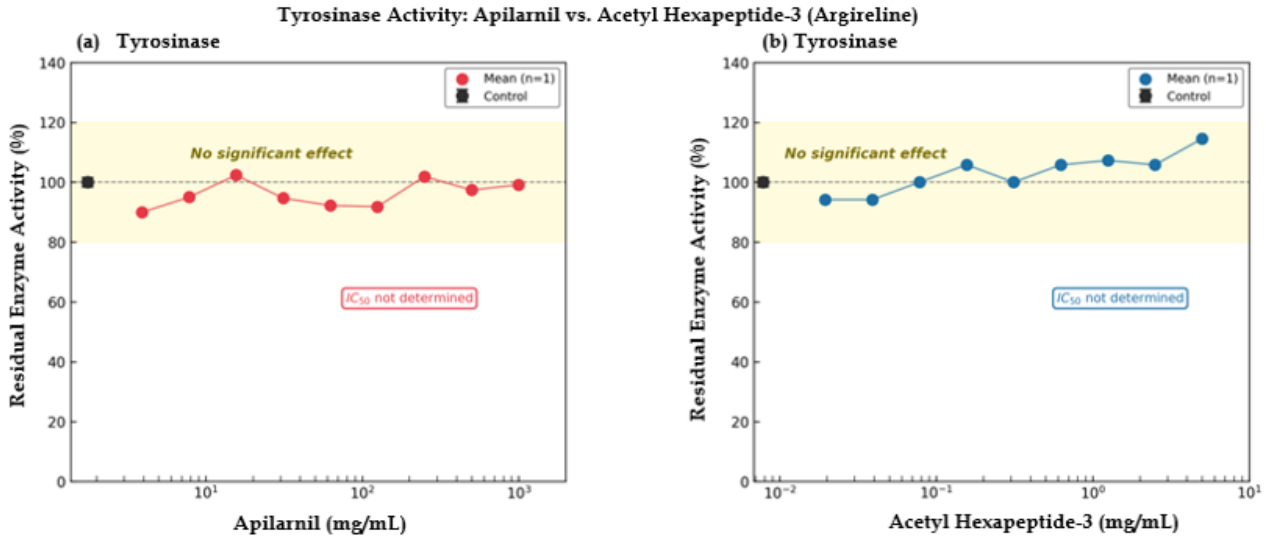

Figure 4. Effects of apilarnil and acetyl hexapeptide-3 on human tyrosinase activity; (a) Apilarnil and (b) Acetyl hexapeptide-3 showed no apparent inhibitory or activating effect on tyrosinase activity under the tested preliminary conditions. Data are presented as a single observation (n=1). Residual enzyme activity is expressed as a percentage of the untreated control.

3.6. Cytotoxicity on HaCaT keratinocytes

The cytotoxic effects of both compounds on HaCaT human keratinocyte cells following 48-hour exposure are presented in Figure 5. Apilarnil maintained cell viability above 90% across all tested concentrations (0.3125–20 mg/mL), with no statistically significant reduction observed relative to the control, indicating a non-cytotoxic profile. In contrast, acetyl hexapeptide-3 exhibited concentration-dependent cytotoxicity, with a CC_{50} value of 5.34% (v/v). As noted in the Methods, this value is expressed relative to the stock solution rather than as an absolute concentration. Cell viability decreased markedly at concentrations above 3.125% (v/v), reaching approximately 2.2% at the highest tested concentration (100% v/v).

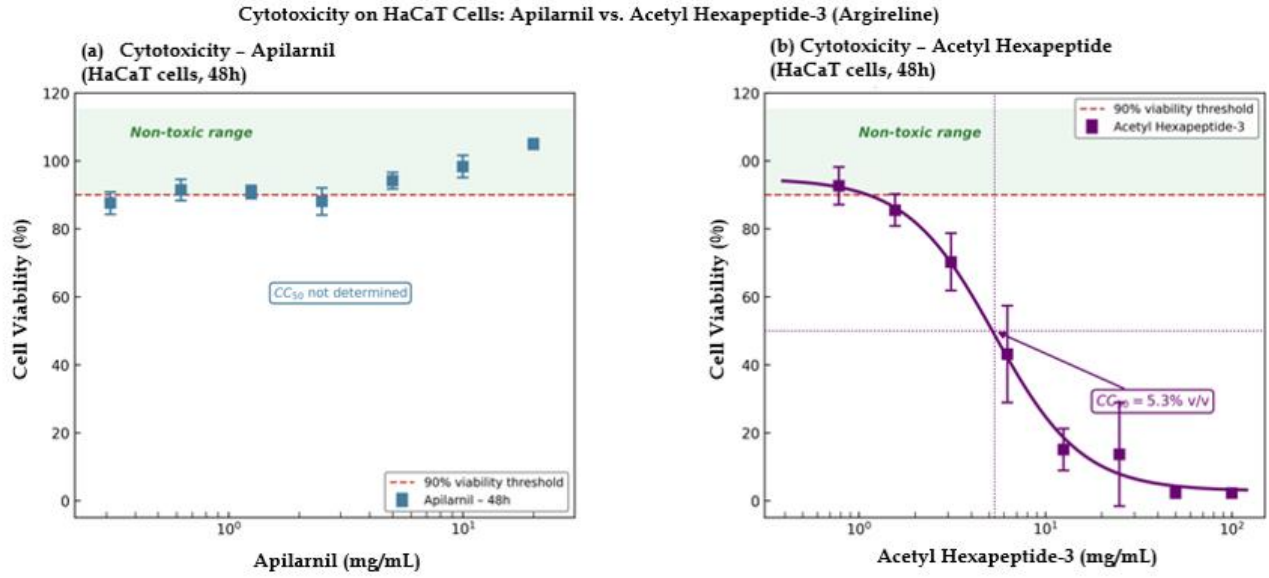

Figure 5. Cytotoxic effects of apilarnil and acetyl hexapeptide-3 on HaCaT human keratinocyte cells following 48-hour exposure; (a) Apilarnil maintained cell viability above the 90% threshold across all tested concentrations (0.3125–20 mg/mL), with CC_{50} not determined, and (b) Acetyl hexapeptide-3 exhibited concentration-dependent cytotoxicity with a CC_{50} of 5.34% v/v. The dashed red line indicates the 90% cell viability threshold. Data are presented as mean ± SD (n=3). Cell viability is expressed as a percentage of the untreated control.

4. DISCUSSION

The present study provides the first direct comparative biological characterization of apilarnil and acetyl hexapeptide-3 with respect to antioxidant capacity, enzyme inhibitory activities relevant to skin aging, and

cytotoxicity on human keratinocytes. The findings reveal contrasting yet complementary bioactivity profiles between the two compounds, with important implications for their respective roles in cosmeceutical applications.

The markedly higher total antioxidant capacity of acetyl hexapeptide-3 relative to apilarnil can be explained by its amino acid composition and peptide structure–activity relationships. The antioxidant activity of peptides is strongly influenced by the nature and proportion of their constituent amino acids; acidic residues such as glutamic acid exhibit free radical scavenging activity due to their electron-donating capacity, while arginine has been reported to inhibit pro-oxidant enzymes and neutralize reactive oxygen species (Wen et al., 2020; Zhu et al., 2022). Given that acetyl hexapeptide-3 consists exclusively of glutamic acid (×2), glutamine (×1), methionine (×1), and arginine (×2), these residues collectively account for its high antioxidant capacity. In addition, the thioether group of methionine contributes to radical scavenging through single-electron transfer mechanisms (Zhu et al., 2022). In contrast, the comparatively lower total antioxidant capacity of apilarnil is consistent with previous reports. Inci et al. (2023) demonstrated that apilarnil exhibits moderate antioxidant activity across multiple assays, while El-Wahed et al. (2024) reported a DPPH IC_{50} value indicative of measurable but limited radical scavenging capacity. The complex composition of apilarnil, which comprises proteins, lipids, carbohydrates, vitamins, and minerals, likely dilutes the contribution of individual antioxidant components compared with a chemically defined peptide. It should be noted that the comparisons in this study were performed on an equal tested-concentration basis rather than after normalization by dry mass, protein content, or active fraction; therefore, the apparent superiority of acetyl hexapeptide-3 may partly reflect its chemically defined, high-purity composition rather than a true difference in intrinsic bioactivity.

The potent and classical sigmoidal inhibition of elastase and collagenase by acetyl hexapeptide-3 aligns with its defined chemical structure. The abundance of charged amino acids (arginine, glutamic acid, glutamine) promotes electrostatic and hydrogen-bond interactions with enzyme active sites (Cruz et al., 2023). The particularly low IC_{50} value observed for collagenase inhibition suggests that acetyl hexapeptide-3 may exert broader anti-aging effects beyond its established mechanism of SNARE complex modulation (Blanes-Mira et al., 2002). While its primary mode of action involves inhibition of neurotransmitter release, the present findings indicate that direct enzyme inhibition may represent an additional mechanism contributing to extracellular matrix preservation. In contrast, apilarnil exhibited a biphasic dose–response profile characterized by enzyme activation at low concentrations and inhibition at higher concentrations. This hormetic response is a well-established phenomenon in biological systems, particularly for natural products and complex biological matrices (Calabrese et al., 2022; Mattson, 2008). Such responses are often interpreted as adaptive or compensatory mechanisms rather than simple toxicity. The observed activation of elastase and collagenase at low concentrations may reflect the stimulatory effects of specific bioactive constituents, including amino acids, hormones, and phenolic compounds such as kaempferol and quercetin identified in apilarnil (Inci et al., 2023). The higher IC_{50} values observed for apilarnil further support its lower potency as a direct enzyme inhibitor, consistent with its heterogeneous composition. It should be noted that porcine pancreatic elastase and bacterial collagenase, while widely used and validated screening enzymes, do not fully replicate the structural and regulatory properties of human skin elastase and matrix metalloproteinases; accordingly, these findings should be regarded as preliminary in vitro screening data rather than direct evidence of human dermal anti-aging efficacy.

In a preliminary single experiment ($n = 1$), neither compound showed an apparent inhibitory effect against human tyrosinase under the tested conditions; given the absence of statistical replication, this finding should not be interpreted as definitive evidence of inactivity and requires confirmation with replicated experiments ($n \geq 3$). The absence of inhibitory activity may be explained by structural requirements for effective tyrosinase inhibition, which typically involve aromatic rings with phenolic or hydroxyl groups capable of interacting with the enzyme's copper-containing active site (Yap & Gan, 2024; Zolghadri et al., 2019). Acetyl hexapeptide-3 lacks aromatic residues entirely, rendering it structurally unsuitable for tyrosinase inhibition. Although apilarnil contains phenolic compounds such as kaempferol and quercetin (Inci et al., 2023), their concentrations may be insufficient to exert measurable effects, or their activity may be masked within the complex biological matrix. Additionally, the use of human tyrosinase in this study is noteworthy, as it differs significantly from mushroom tyrosinase commonly used in screening assays, which may contribute to discrepancies in reported inhibitory activities (Song et al., 2022).

The cytotoxicity findings represent one of the most practically relevant outcomes of this study. Apilarnil exhibited no cytotoxic effects across all tested concentrations, maintaining cell viability above 90%, which is consistent with its established safety profile and previous experimental findings (Elashal et al., 2024). This supports its suitability as a safe ingredient for topical cosmeceutical formulations. In contrast, acetyl hexapeptide-3 demonstrated concentration-dependent cytotoxicity, with a CC_{50} of 5.34% (v/v), although the lack of a corresponding absolute concentration limits direct comparison with the enzyme inhibition IC_{50} values reported in mg/mL. However, this finding should be interpreted in the context of typical formulation concentrations. Previous studies have shown that cytotoxic effects of Argireline occur at concentrations significantly higher than those used in cosmetic applications (Grosicki et al., 2014). Therefore, while cytotoxicity is evident under in vitro conditions at high concentrations, it is unlikely to pose a risk under standard usage conditions.

Overall, the findings of this study indicate that acetyl hexapeptide-3 and apilarnil exhibit complementary rather than competing biological activities. Acetyl hexapeptide-3 demonstrates superior potency as an antioxidant and enzyme inhibitor, whereas apilarnil offers a favorable safety profile, hormetic biological responses, and a multifunctional composition that may warrant future investigation in combined formulations. Future studies investigating combined formulations and in vivo validation of these findings would provide valuable insights into their potential applications in dermocosmetics.

5. CONCLUSIONS

This study provides the first direct comparative biological characterization of apilarnil and acetyl hexapeptide-3, two structurally and compositionally distinct compounds with demonstrated relevance in cosmeceutical research. The findings confirm that these compounds exhibit contrasting yet potentially complementary bioactivity profiles.

Acetyl hexapeptide-3 displayed markedly higher antioxidant capacity and potent, concentration-dependent inhibition of elastase (IC_{50} = 1.534 mg/mL) and collagenase (IC_{50} = 0.00811 mg/mL), indicating that direct enzyme inhibition may represent an additional mechanism contributing to its anti-aging effects beyond its established neurotransmitter-inhibitory activity. In contrast, apilarnil demonstrated a biphasic (hormetic) dose–response pattern, characterized by enzyme activation at low concentrations and inhibition at higher concentrations, alongside an excellent safety profile with no detectable cytotoxicity in HaCaT keratinocytes across all tested concentrations.

Neither compound showed an apparent inhibitory effect against human tyrosinase within the tested ranges in a single preliminary experiment (n = 1); due to the lack of statistical replication, this observation should be considered exploratory rather than conclusive, and requires confirmatory replication, highlighting the structural specificity required for effective inhibition of this enzyme and emphasizing the importance of using human-derived enzyme systems in cosmeceutical screening. The distinct cytotoxicity profiles, with apilarnil showing no toxicity and acetyl hexapeptide-3 demonstrating a CC_{50} of 5.34% (v/v), further differentiate these compounds in terms of formulation safety.

Overall, the results support the concept that apilarnil and acetyl hexapeptide-3 are not competing but complementary bioactive agents, integrating potent enzyme inhibition with a favorable safety profile and multifunctional biological activity. Their combined use in optimized formulations may warrant future investigation. Future studies should focus on in vivo validation, formulation optimization, and direct combination assays to evaluate potential synergistic interactions between these compounds.

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AUTHOR CONTRIBUTION

S.D.K.: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration

M.K.: Resources, Writing – review & editing

DECLARATIONS

Ethics Approval and Consent to Participate

This study did not involve any human participants or animals. Therefore, ethics committee approval was not required.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

Amano, S. (2009). Possible involvement of basement membrane damage in skin photoaging. *Journal of Investigative Dermatology Symposium Proceedings, 14*(1), 2–7.

Asoro, N. J., Guevara, M. A., Berendt, K., Zijlstra, R., & Shoveller, A. K. (2017). Digestibility is similar between commercial diets that provide ingredients with different perceived glycemic responses. *Veterinary Sciences, 4*(4), 54–65.

Blanes-Mira, C., Clemente, J., Jodas, G., Gil, A., Fernández-Ballester, G., Ponsati, B., et al. (2002). A synthetic hexapeptide (Argireline) with antiwrinkle activity. *International Journal of Cosmetic Science, 24*(5), 303–310.

Calabrese, E. J., Dhawan, G., Kapoor, R., Agathokleous, E., & Calabrese, V. (2022). Hormesis: Wound healing and keratinocytes. *Pharmacological Research, 183*, 106393.

Crous, C., Pretorius, J., & Petzer, A. (2024). Overview of popular cosmeceuticals in dermatology. *Skin Health and Disease, 4*(2), e340.

Cruz, A. M., Gonçalves, M. C., Marques, M. S., Veiga, F., Paiva-Santos, A. C., & Pires, P. C. (2023). In vitro models for anti-aging efficacy assessment: A critical update in dermocosmetic research. *Cosmetics, 10*(2), 66.

Elashal, M. H., El-Wahed, A. A. A., Mohamed, M. A., Hamad, R., Eldaim, M. A. A., Khalifa, S. A. M., et al. (2024). Apilarnil ameliorates bisphenol A-induced testicular toxicity in adult male rats via improving antioxidant potency and PCNA expression. *Reproductive Toxicology, 125*, 108570.

El-Wahed, A. A. A., Khalifa, S. A. M., Aldahmash, B., Zhang, H., Du, M., Zhao, C., et al. (2024). Exploring the chemical constituents and nutritive potential of bee drone (apilarnil): Emphasis on antioxidant properties. *Chemistry & Biodiversity, 21*(5), e202400085.

Gorouhi, F., & Maibach, H. I. (2009). Role of topical peptides in preventing or treating aged skin. *International Journal of Cosmetic Science, 31*(5), 327–345.

Grosicki, M., Latacz, G., Szopa, A., Cukier, A., & Kieć-Kononowicz, K. (2014). The study of cellular cytotoxicity of argireline – an anti-aging peptide. *Acta Biochimica Polonica, 61*(1), 29–32.

Hussein, R. S., Dayel, S. B., Abahussain, O., & El-Sherbiny, A. A. (2025). Influences on skin and intrinsic aging: Biological, environmental, and therapeutic insights. *Journal of Cosmetic Dermatology, 24*(2), e16688.

Inci, H., Izol, E., Yilmaz, M. A., Ilkaya, M., Bingöl, Z., & Gülçin, I. (2023). Comprehensive phytochemical content by LC/MS/MS and anticholinergic, antiglaucoma, antiepilepsy, and antioxidant activity of apilarnil (drone larvae). *Chemistry & Biodiversity, 20*(10), e202300654.

Jensen, A. B., Evans, J., Jonas-Levi, A., Benjamin, O., Martinez, I., Dahle, B., Roos, N., Lecocq, A., & Foley, K. (2019). Standard methods for *Apis mellifera* brood as human food. *Journal of Apicultural Research, 58*(2), 1–28.

Jiratchayamaethasakul, C., Ding, Y., Hwang, O., Im, S. T., Jang, Y., Myung, S. W., et al. (2020). In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. *Fisheries and Aquatic Sciences, 23*, 6.

Khvotchev, M., & Soloviev, M. (2022). SNARE modulators and SNARE mimetic peptides. *Biomolecules, 12*(12), 1779.

Krutmann, J., Schikowski, T., Morita, A., & Berneburg, M. (2021). Environmentally-induced (extrinsic) skin aging: Exposomal factors and underlying mechanisms. *Journal of Investigative Dermatology, 141*(4), 1096–1103.

Makrantonaki, E., & Zouboulis, C. C. (2007). Molecular mechanisms of skin aging. *Annals of the New York Academy of Sciences, 1119*(1), 40–50.

Martic, I., Jansen-Dürr, P., & Cavinato, M. (2022). Effects of air pollution on cellular senescence and skin aging. *Cells, 11*(14), 2220.

Mattson, M. P. (2008). Hormesis defined. *Ageing Research Reviews, 7*(1), 1–7.

Pillaiyar, T., Manickam, M., & Namasivayam, V. (2017). Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry, 32*(1), 403–425.

Poljšak, B., & Dahmane, R. (2012). Free radicals and extrinsic skin aging. *Dermatology Research and Practice*, 2012, 135206.

Roulier, B., Rush, I., Lazinski, L. M., Pérès, B., Olleik, H., Royal, G., et al. (2023). Resorcinol-based hemiindigoid derivatives as human tyrosinase inhibitors and melanogenesis suppressors. *European Journal of Medicinal Chemistry, 246*, 114972.

Sidor, E., & Dzugan, M. (2020). Drone brood homogenate as natural remedy for treating health care problem: A scientific and practical approach. *Molecules, 25*(23), 5699–5713.

Song, Y., Chen, S., Li, L., Zeng, Y., & Hu, X. (2022). The hypopigmentation mechanism of tyrosinase inhibitory peptides derived from food proteins. *Molecules, 27*(9), 2710.

Wang, Y., Wang, M., Xiao, S., Pan, P., Li, P., & Huo, J. (2013). The anti-wrinkle efficacy of argireline, a synthetic hexapeptide, in Chinese subjects: A randomized, placebo-controlled study. *American Journal of Clinical Dermatology, 14*(2), 147–153.

Wen, C., Zhang, J., Zhang, H., Duan, Y., & Ma, H. (2020). Plant protein-derived antioxidant peptides: Isolation, identification, mechanism of action and application in food systems: A review. *Trends in Food Science & Technology, 105*, 308–322.

Yap, P. G., & Gan, C. Y. (2024). Tyrosinase inhibitory peptides: Structure–activity relationship study on peptide chemical properties, terminal preferences and intracellular regulation of melanogenesis signaling pathways. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1868*(1), 130503.

Yücel, B., Şahin, H., Yıldız, O., & Kolayli, S. (2019). Bioactive components and effect mechanism of apilarnil. *Journal of Animal Production, 60*(2), 125–130.

Zachara, A., Galkowska, D., & Juszczak, L. (2017). Method validation and determination of polycyclic aromatic hydrocarbons in vegetable oils by HPLC-FLD. *Food Analytical Methods, 10*(4), 1078–1086.

Zhu, Y., Lao, F., Pan, X., & Wu, J. (2022). Food protein-derived antioxidant peptides: Molecular mechanism, stability and bioavailability. *Biomolecules, 12*(11), 1622.

Zolghadri, S., Bahrami, A., Khan, M. T. H., Muñoz-Muñoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry, 34*(1), 279–309.