Tyrosinase Inhibitory Properties of D-Qonamide™

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***Abstract*—Tyrosinase is a key enzyme in melanin biosynthesis, catalyzing reactions that produce melanin in melanocytes and contributing to pigmentation in mammals and plants. This enzyme overactivity is a major contributing factor in hyperpigmentation disorders including as melasma, lentigo, and freckles. Current study has focused on combining active substances under the formulation D-Qonamide™: Dong Quai (Angelica sinensis) extract, grape (Vitis vinifera) skin extract (resveratrol), astragalus (Astragalus membranaceus) extract, and nicotinamide, to efficiently inhibit this enzyme, resulting in potential treatments for controlling various skin problems. The objective of this study is to evaluate the efficacy of these ingredients in addressing anti-tyrosinase activity. D-Qonamide™ were evaluated for their in vitro tyrosinase inhibitory effect. The enzymatic assay of D-Qonamide™ showed concentration-dependent tyrosinase inhibition, with activity reduced by 35.240±1.092% to 70.989±0.790% at concentrations of 2.00–10.00 mg/mL. Its IC50 was 4.948±0.126 mg/mL, indicating moderate inhibitory potency. In contrast, Kojic acid had a much lower IC50 of 0.674±0.002 mg/mL, making it approximately seven times more effective than D-Qonamide™. Together, these findings suggest that these ingredients, either individually or in combination, offer substantial potential for applications in nutraceuticals, cosmeceuticals, and functional health products targeting pigmentation and skin health.**

***Keywords*—tyrosinase, antioxidant, polyphenol, melanin, Dong quai**

I. Introduction

Melanin is a dark pigment found in a variety of organisms, providing coloration in skin, hair, feathers, and eyes in animals. In mammals, eumelanin (brown-to-black) and pheomelanin (yellow-to-pink) are the two primary types. Melanin is synthesized in melanocytes through melanogenesis, a complex enzymatic process influenced by intrinsic and extrinsic factors such as hormones and Ultraviolet (UV) radiation. While melanin protects against UV damage, its overproduction can lead to hyperpigmentation, aesthetic concerns, and skin cancer. Tyrosinase, a copper-containing enzyme, plays a key role in melanin synthesis by catalyzing the conversion of L-tyrosine to L-DOPA and then to dopaquinone, which forms eumelanin and pheomelanin. Inhibiting tyrosinase is a major strategy for managing melanin-related disorders, with applications in cosmetics, particularly skin-lightening products. Common inhibitors include arbutin, azelaic acid, hydroquinone, and kojic acid, but these pose health risks such as toxicity and carcinogenicity. Safer, more effective inhibitors are needed for both therapeutic and cosmetic purposes. The signaling mechanism of the ligands is illustrated in Fig. 1. In addition to their role as substrates in melanogenesis, L-tyrosine and L-DOPA act as bioregulators, modulating melanogenesis and other cellular functions. Excess melanin accumulation beneath the skin contributes to disorders, making tyrosinase inhibition a critical target for treatment. Tyrosinase inhibitors also find use in food processing and environmental applications [1]. Recent advancements utilize tyrosinase to produce innovative materials like organic semiconductors, photovoltaic devices, and industrial biocatalysts, highlighting its expanding scientific and industrial significance.

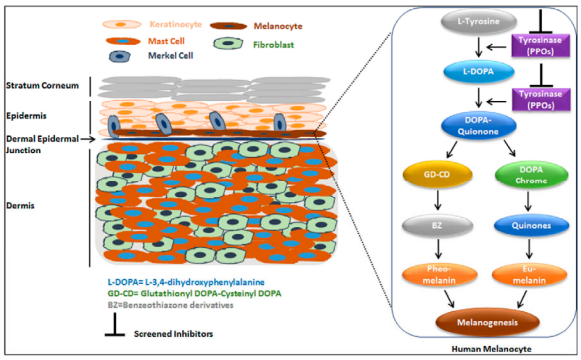

Fig. 1. The basic mechanistic pathway of melanin in the melanogenesis [1].

Tyrosinase is a key enzyme involved in melanin production, playing a central role in skin pigmentation and associated disorders. Inhibiting tyrosinase activity has become a major focus in dermatology and cosmetology, particularly for managing hyperpigmentation, melasma, and uneven skin tones [1]. Various natural extracts and bioactive compounds have been identified for their dual antioxidant and tyrosinase-inhibitory properties, making them ideal candidates for pigmentation control and skin health improvement. Among these, Dong Quai Extract (Angelica sinensis), Grape Skin Extract, Astragalus membranaceus Extract, and Nicotinamide (niacinamide) stand out for their proven efficacy and diverse mechanisms of action. Although extensively studied individually, their combined potential as a synergistic formulation remains largely unexplored [2–3].

Dong Quai Extract has demonstrated robust antioxidant activity and promising potential for tyrosinase inhibition. With its high Total Phenolic Content (3330.3 µmol Trolox Equivalent (TE) per 100 g dry mass) and bioactive components like ferulic acid and rutin, the extract effectively scavenges free radicals and mitigates oxidative stress [2–3]. These phenolic compounds are known to interact with tyrosinase enzymes, reducing melanin synthesis. Moreover, the antioxidant activity and components of Dong Quai Extract (Angelica sinensis) are shown in Table 1. Similarly, Grape Skin Extract, rich in polyphenols such as resveratrol, anthocyanins, and quercetin, exhibits significant antioxidant properties while also serving as a potent inhibitor of tyrosinase. Comparative studies reveal its ability to match or surpass the activity of synthetic inhibitors, emphasizing its dual benefits for health and cosmetic applications [4–6].

Table 1. Antioxidant activity and components of Dong Quai extract (Angelica sinensis) [2–3]

Parameter	Method	Value	Details
Antioxidant Activity	CUPRAC	1330.45 µmol TE/100 g DM	Measures cupric ion-reducing antioxidant capacity.
	FRAP	1813.9 µmol TE/100 g DM	Measures ferric ion-reducing antioxidant parameter, showing higher antioxidant potential.
	Fluorescence	35.96–304.6 µmol TE/100 g DM	Fluorescence-based assay for reactive species.
Total Phenolic Content (TPC)	Folin–Ciocalteu	3330.3 µmol TE/100 g DM	High TPC indicates strong antioxidant capacity.
Key Phenolic Acids	HPLC	Ferulic Acid: 21.83 mg/100 g DM	Contributes significantly to antioxidant and potential tyrosinase inhibition.
		Caffeic Acid, Chlorogenic Acid (Detected)	Additional phenolic acids with antioxidative properties.
Key Flavonoids	HPLC	Rutin: 3.32 mg/100 g DM	Predominant flavonoid; associated with antioxidant and tyrosinase inhibition activity.
		Quercetin, Kaempferol (Detected)	Complementary flavonoids enhancing antioxidant capacity.
Tyrosinase Inhibition Potential	Inferred from phenolic & flavonoid content	High (Potential)	Phenolic acids (e.g., ferulic acid) and flavonoids (e.g., rutin) are known tyrosinase inhibitors, though specific data was not directly provided.
Applications		Dietary supplements, cosmetics	Suitable for anti-aging, skin whitening, oxidative stress reduction, and general health maintenance.

Astragalus membranaceus Extract adds another dimension with its phenolic-rich profile, including compounds like calycosin, which has shown a superior tyrosinase-inhibitory effect compared to kojic acid. Its antioxidant activity, validated through assays such as DPPH and FRAP, correlates strongly with its tyrosinase-inhibitory potential. Calycosin not only reduces melanin synthesis in melanocytes but does so without inducing cytotoxicity, highlighting its suitability for safe and effective pigmentation control [7–8]. On the other hand, Nicotinamide (niacinamide) directly inhibits tyrosinase and slows the transfer of melanosomes from melanocytes to keratinocytes, further enhancing its skin-whitening properties. As a vitamin B3 derivative, nicotinamide also plays a critical role in cellular redox balance and UV protection, making it a multifunctional agent in skincare formulations [9–10].

Despite their well-documented individual benefits, the collective potential of these ingredients remains underexplored. Current research largely focuses on their isolated effects, leaving a gap in understanding their synergistic mechanisms and cumulative efficacy in tyrosinase inhibition and oxidative stress reduction. Investigating their combined action could yield new insights into their biochemical interactions, optimize formulations for pigmentation control, and enhance their application in cosmetics and therapeutics.

Studying the synergistic effects of Dong quai extract, grape skin extract, astragalus membranaceus extract, and nicotinamide could lead to the development of advanced solutions for skin health and pigmentation disorders. Current study has focused on combining active substances under the formulation D-Qonamide™ to efficiently inhibit this enzyme, resulting in potential treatments for controlling various skin problems. The objective of this study is to evaluate the efficacy of these ingredients in addressing anti-tyrosinase activity. Their diverse bioactive compounds and complementary mechanisms provide a robust foundation for comprehensive research. Addressing these gaps would not only expand scientific understanding but also contribute to innovative approaches for managing skin pigmentation, aging, and oxidative stress, offering both therapeutic and cosmetic advancements.

II. Materials and Methods

A. Materials

D-Qonamide™ was procured from Beyond Laboratory (Thailand) Co., Ltd., Bangkok, Thailand, while all chemicals used in the analysis were of analytical reagent (AR) grade.

B. Inhibition of Tyrosinase Activity Assay

1) Preparation for solution test

To prepare the solutions for the test, both standard and sample solutions were prepared as follows: Kojic acid (Sigma-Aldrich, MO, US) was used as the standard and prepared in varying concentrations of 0.3, 0.4, 0.5, 0.6, and 0.7 mg/mL in phosphate buffer. The sample solution, D-Qonamide™ (Beyond Laboratory (Thailand) Co. Ltd., Bangkok, Thailand), was prepared at concentrations of 2, 4, 6, 8, and 10 mg/mL in Dimethyl Sulfoxide (DMSO). For the testing solutions, a 50 mM phosphate buffer was prepared by dissolving one Phosphate-Buffered Saline (PBS) tablet in 40 mL of distilled water, followed by thorough mixing using a magnetic stirrer. Tyrosinase enzyme (333 unit/mg) was prepared by dissolving 0.5 mg of tyrosinase in 1 mL phosphate buffer to create a stock solution, which was then diluted to a working solution using 0.093 mL of stock solution mixed with 0.907 mL of phosphate buffer. Additionally, a 12 mM solution of L-Dopa was prepared by dissolving 0.2366 g of L-Dopa in 100 mL of distilled water. These solutions were used to perform the enzymatic assays.

2) Assay of inhibition

The tyrosinase inhibitory activity assay was conducted using a modified method based on Muddathir [1]. The reaction mixture components and volumes are detailed in Table 2. Briefly, 70 µL of the sample solution was added to a 96-well plate, followed by 30 µL of tyrosinase enzyme solution (333 unit/mL in 50 mM phosphate buffer, pH 6.5) and 110 µL of substrate solution (either 2 mM L-tyrosine or 12 mM L-DOPA). The mixture was incubated at 37 °C for 30 minutes, and the absorbance at 510 nm was measured using a microplate reader. The percent inhibition of tyrosinase activity was calculated for sample concentrations of 2, 4, 6, 8, and 10 µg/mL using Eq. (1). Samples showing enzyme inhibition activity of 50% or higher were expressed as IC_{50} values. Kojic acid was used as a positive control for comparison.

$$\text{Inhibition (\%)} = \frac{(A-B)-(C-D)}{(A-B)} \times 100 \quad (1)$$

where
A = 100 % of tyrosinase
B = Background of 100 % w/v of tyrosinase
C = standard reaction / sample reaction
D = Background of standard reaction / Background of sample reaction.

Table 2. Amount of solution that use for reaction of anti-tyrosinase

Sample test	Phosphate Buffer (µl)	Kojic Acid (µl)	D-Qonamide™ (µl)	Enzyme Tyrosinase (µl)	L-Dopa (µl)
100 % tyrosinase	70	-	-	30	110
Background of tyrosinase	100	-	-	-	110
Standard	60	10	-	30	110
Background of Standard	90	10	-	-	110
D-Qonamide™	60	-	10	30	110
Background of D-Qonamide™	90	-	10	-	110

III. Result and Discussion

A. Tyrosinase Inhibitory Activity

The enzymatic assay of D-Qonamide™ revealed a concentration-dependent inhibition of tyrosinase activity, as shown in Fig. 2. Specifically, concentrations of 2.00, 4.00, 6.00, 8.00, and 10.00 mg/mL inhibited tyrosinase by 35.240±1.092%, 49.201±1.307%, 56.543±2.083%, 64.532±0.948%, and 70.989±0.790%, respectively. These results indicate that D-Qonamide™ effectively inhibits tyrosinase activity in a dose-dependent manner, with greater concentrations leading to increased inhibition. This suggests its potential utility in formulations aimed at reducing melanin production, positioning it as a promising candidate for skin-lightening and hyperpigmentation treatment products.

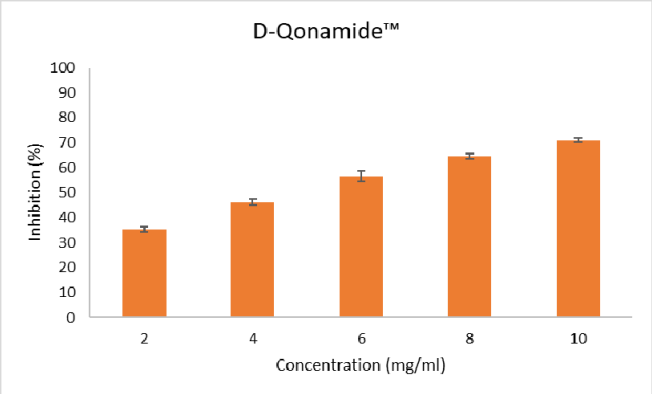

Fig. 2. Inhibition of tyrosinase activity with difference concentration of D-Qonamide™.

The tyrosinase inhibitory effect of D-Qonamide™ was demonstrated by its half-maximal inhibitory concentration (IC_{50}) as shown in Fig. 3, which was found to be 4.948±0.126 mg/mL. In comparison, the IC_{50} of the control sample, Kojic acid, was significantly lower at 0.674±0.002 mg/mL, indicating that Kojic acid is approximately seven times more effective as a tyrosinase inhibitor compared to D-Qonamide™. This significant difference in IC_{50} values highlights that, while D-Qonamide™ has notable inhibitory activity, Kojic acid is a more potent tyrosinase inhibitor at lower concentrations.

D-Qonamide™ exhibits properties consistent with findings on other tyrosinase inhibitors, particularly in terms of dose-dependent inhibition, natural alternatives, synergistic potential, and non-cytotoxicity. Like many inhibitors, D-Qonamide™ shows increasing efficacy with higher concentrations, similar to thiosemicarbazones, which have demonstrated up to 41 times the potency of kojic acid. Its alignment with naturally occurring inhibitors, such as flavonoids and aromatic carboxylic acids, which often exhibit IC_{50} values comparable to kojic acid, underscores its potential in health-promoting applications [11]. Furthermore, D-Qonamide™'s suitability as a dietary supplement aligns with research supporting the synergistic use of tyrosinase inhibitors with antioxidants and skin-brightening agents for enhanced skin health. Importantly, its non-cytotoxicity at effective concentrations reflects the safety benchmarks established in studies, strengthening its promise as a safe and effective ingredient for health and cosmetic applications [12]. The findings suggest that D-Qonamide™ aligns well with key principles in tyrosinase inhibitor research, including dose-dependence, natural alternatives, synergistic formulation potential, and non-cytotoxicity. Its application in dietary supplements and skin health products could be further supported by combining it with complementary ingredients for enhanced outcomes.

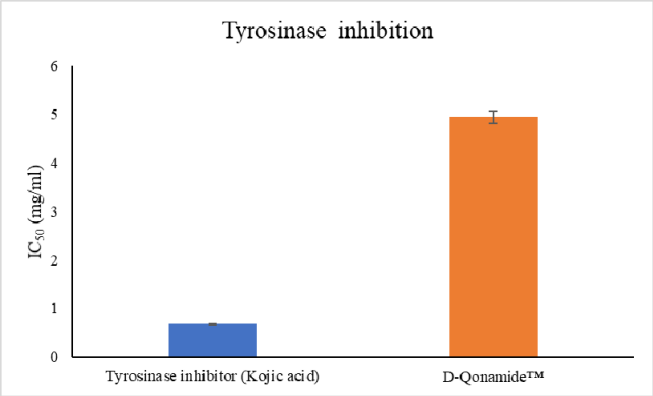

Fig. 3. Comparison of Kojic acid and D-Qonamide on half-maximal inhibitory concentration (IC_{50}).

The study on D-Qonamide™ reveals its concentration-dependent inhibition of tyrosinase activity, making it a promising candidate for skin-lightening and hyperpigmentation treatments. At higher concentrations, D-Qonamide™ effectively reduces tyrosinase activity, although its half-maximal Inhibitory Concentration (IC50) is significantly higher compared to the more potent Kojic acid. Despite this, D-Qonamide™ aligns with natural alternatives like flavonoids and aromatic carboxylic acids, which are commonly used in skin health products. Its non-cytotoxicity at effective concentrations further supports its potential as a safe ingredient for dietary supplements and skincare formulations. The findings suggest that while D-Qonamide™ may not yet surpass traditional inhibitors in potency, its synergistic potential, natural profile, and safety make it a valuable addition to health and cosmetic applications.

IV. Conclusion

D-Qonamide™ demonstrates promising dose-dependent tyrosinase inhibitory activity, making it a viable candidate for skin-lightening and hyperpigmentation treatment products. Although its IC50 is higher compared to Kojic acid, D-Qonamide™'s non-cytotoxicity and alignment with natural tyrosinase inhibitors reinforce its safety and potential as a natural alternative. These properties position it as a valuable ingredient for dietary supplements and skincare formulations, especially when combined with synergistic agents to enhance efficacy. Further studies, including in vivo and clinical trials, are recommended to optimize its potency, validate its effectiveness, and establish its commercial potential in health and cosmetic applications.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Chanin Leksahakhun is responsible for conceptualization, conducting research, and drafting the initial version. Laichheang Yort focuses on conceptualization, validation, formal analysis, reviewing, and editing the writing. All authors had approved the final version.

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