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# Biological Potentials of *Etlingera elatior* Inflorescences Extracted via Microwave-Assisted Extraction for Cosmetic Applications

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#### Abstract

Etlingera elatior, widely known as Torch ginger, is a member of the Zingiberaceae family. It is recognized for its medicinal benefits, such as aiding the healing of skin conditions, alleviating flatulence, and enhancing blood circulation. Therefore, this research aimed to investigate the antioxidant, tyrosinase inhibitory, and anti-inflammatory activities of the extracts, along with the total phenolic and flavonoid contents, from three varieties of Torch ginger inflorescences: red, pink, and white. Each type of inflorescence was extracted using microwave-assisted extraction (MAE) with different ethanol concentrations (100%, 80%, and 50% v/v). The results showed that the 50% ethanolic extract of the red inflorescence exhibited strong antioxidant activity, with the highest DPPH and ABTS radical scavenging effects. Moreover, this extract contained the highest concentrations of total phenolics and flavonoids, measuring 102.7 mg gallic acid equivalents (GAE)/g of extract and 56.7 mg catechin/g of extract, respectively. Regarding melanin production, the 100% ethanolic extract of the red inflorescence showed the greatest reduction in melanin production in B16F10 melanoma cells and the highest tyrosinase inhibitory activity. Furthermore, when anti-inflammatory activity was assessed based on nitric oxide inhibition in RAW264.7 macrophage cells, the 100% ethanolic extract of the red inflorescence demonstrated significant effectiveness without any signs of cytotoxicity, with an IC<sub>50</sub> value of 40.4 μg/mL. Overall, MAE of Torch ginger inflorescences using different ethanol concentrations yielded a phenolic and flavonoid rich extract with potent antioxidant, anti-tyrosinase, and anti-inflammatory activities, positioning it as a promising candidate for next-generation skin-brightening and anti-inflammatory cosmeceutical formulations.

**Keywords:** Etlingera elatior; torch ginger; anti-oxidant; anti-inflammation; anti-tyrosinase; melanin production; microwave-assisted extraction

# 1. Introduction

Nowadays, people are increasingly interested in health and beauty, particularly in food and cosmetics derived from herbal plants because of their medicinal properties and nutritional value. The cosmetic industry is a major source of revenue for Thailand. Currently, the cosmetic market trend emphasizes natural ingredients, as they are considered safe and effective, often matching or even surpassing the efficacy of chemical-based cosmetics. Additionally,

awareness of the dangers, side effects, and toxicity of synthetic chemicals has grown (Ustymenko, 2023). As a result, research on various herbal plants has increased. Thailand has abundant natural resources and a rich tradition of using natural substances for health and beauty benefits, a practice rooted in local wisdom for generations. Most herbal plant components contain antioxidants, which play a crucial role in counteracting oxidative stress caused by free radicals (Pradubyat et al., 2025). In addition to

oxidative stress, skin pigmentation is another important biological process influenced by both internal and external factors (Klaisung et al., 2024). It serves as a crucial physiological defense mechanism, protecting skin cells from the harmful effects of ultraviolet (UV) radiation by preventing UV-induced DNA damage (Aiamsaard et al., 2024) and reducing oxidative stress. However, excessive melanin production can lead to hyperpigmented skin disorders such as melasma, freckles (ephelides), age spots, melanoderma, solar lentigo, and postinflammatory hyperpigmentation, which can appear on the face or body. Facial hyperpigmentation, in particular, often affects individuals' confidence and emotional well-being and, in some cases, may contribute to anxiety or depression (Klaisung et al., 2024). Antioxidants help slow down or prevent oxidation reactions in the body, protecting biomolecules such as proteins and enzymes. This, in turn, helps prevent wrinkles, firm the skin, and inhibit oxidative damage. Living organisms have natural defense systems to protect cells and tissues from free radical damage, consisting of various antioxidants with distinct functions (Sulaiman et al., 2013). Most antioxidants belong to the phenolic and flavonoid groups, with flavonoids being particularly abundant in the flowers of many herbal plants (Prabawati et al., 2021). This research focuses on studying the antioxidant and anti-inflammatory properties of Torch inflorescences, along with their anti-tyrosinase activity related to melanin production.

Microwave-assisted extraction (MAE) is a widely used method for extracting bioactive compounds (Suksaeree & Monton, 2024; Suksaeree et al., 2024). Several factors influence the efficiency of MAE, including the solvent system and its ratio to herbal powder, microwave power and temperature, extraction duration and number of extraction cycles, surface area, and stirring of the substances (Pogorzelska-Nowicka et al., 2024). MAE is a simple, convenient, and rapid extraction technique that enhances the yield of target compounds. It also reduces the amount of solvent required, thereby lowering extraction costs (Wang et al., 2025). Additionally, MAE shortens extraction time compared to conventional methods such as fermentation or solvent percolation, which do not involve heat. These traditional techniques often result in significant losses of key compounds, require large amounts of solvents, and take longer to complete (Patthamasopasakul et al., 2024). MAE can be used to extract various natural compounds, including glycosides, alkaloids, carotenoids, terpenoids, polyphenols, and essential oils (Pogorzelska-Nowicka et al., 2024). However, this method has some limitations. It is particularly suitable for extracting small phenolic compounds, such as phenolic acids (e.g., gallic acid and ellagic acid), quercetin, isoflavones, and transresveratrol (Afoakwah et al., 2012; Niu et al., 2020).

Etlingera elatior (Jack) R.M. Smith or Torch ginger is classified as a single species; however, it exhibits several bract-color morphotypes both in cultivation and the wild (Chan et al., 2011a). Torch ginger has been cultivated for a long time in southern Thailand, where it is well known for its striking shape, vibrant colors, and durability. It is a summerblooming flower that produces a large number of blossoms. Torch ginger shares medicinal properties with ginger and galangal, possessing a bitter and slightly sour aroma along with a spicy taste. It has been traditionally used to alleviate hives, skin flatulence, and bloating. constituent analyses of Torch ginger have identified important compounds belonging to the phenolic and flavonoid groups in its leaves, flowers, and rhizomes, all of which exhibit strong antioxidant properties (Chan et al., 2011a). Additionally, Torch ginger has demonstrated antibacterial properties (found in its essential oils and leaves), hepatoprotective activity (from its inflorescences), tyrosinase inhibitory activity (from its leaves), and cancer cell inhibitory activity (from its rhizomes) (Juwita et al., 2018; Chan et al., 2011b).

Previous research has found that freeze-dried Torch ginger inflorescence extracts possess potential wound healing and anti-aging effects and are a good source of natural phenolic compounds (Sinsuebpol et al., 2023).

Given these beneficial properties, Torch ginger is an interesting candidate for further study on its antioxidant and anti-inflammatory activities. In addition, there are no published reports on the bioactivities of Torch ginger inflorescence extracts from three different varieties, red, pink, and white, obtained using MAE. This study aims to evaluate the antioxidant, anti-inflammatory, and anti-tyrosinase properties of Torch ginger inflorescences extract with the goal of building foundation of knowledge to support future product development and applications. If the inflorescence extracts demonstrate these beneficial activities, they could serve as promising ingredients in cosmetic formulations, including creams, skincare products, and dietary supplements.

# 2. Objectives

This study aims to evaluate the total phenolic and flavonoid contents of Torch ginger inflorescence extracts obtained through MAE. Additionally, it investigates the antioxidant, anti-inflammatory, and anti-tyrosinase activities of these extracts in relation to melanin production across different color varieties of Torch ginger inflorescences.

# 3. Materials and Methods

#### 3.1 Plant Material and Extraction

Torch ginger inflorescences were collected at full bloom from three different varieties: red, pink, and white. The inflorescences were first washed, and then dried in a hot air oven at 50°C. After drying, the inflorescences were ground into a fine powder using a grinder and passed through a No. 30 sieve for uniformity (Figure 1). The powdered inflorescences were stored in vacuum-sealed bags in a cool place, protected from light.

For extraction, 20 g of each Torch ginger inflorescence powder was separately extracted using a microwave oven (Sharp, model R-270, Japan) with three different ethanol concentrations, including 100%, 80% and 50% v/v (200 mL each). The MAE process was carried out using 850 W of electrical power, with the microwave system set to power on for 30 seconds, followed by a power-off period of 1 minute. This cycle was repeated for three rounds. The extract was then filtered, and the remaining powder was re-extracted using the same method, with the process performed in triplicate. The extracts were pooled and dried using a rotary evaporator (Buchi, model R215, Switzerland) under reduced pressure at a temperature of 40-45°C. This process resulted in a total of nine Torch ginger inflorescence extracts. The extracts were collected, weighed, and recorded. These extracts were then used for testing their antioxidant and anti-inflammatory properties.

# 3.2 Phytochemical Analysis

#### 3.2.1 Total Phenolic Content

The total phenolic content (TPC) was determined as described by Settharaksa et al. (2014). For the analysis of TPC in Torch ginger extracts, gallic acid was used as the standard. A stock solution of gallic acid (1 mg/mL) was prepared by dissolving it in absolute ethanol. The experiment involved pipetting 12.5  $\mu L$  of the sample extract and 50  $\mu L$  of distilled water into a 96-well plate, followed by the addition of 12.5  $\mu L$  Folin-Ciocalteu reagent. The mixture was shaken well and incubated for 6 minutes. Then, 125

 $\mu L$  of 7% sodium carbonate solution (w/v) and 100  $\mu L$  of distilled water were added, mixed thoroughly, and incubated at room temperature for 90 minutes. The absorbance was measured at 760 nm using a microplate reader (Bio-Rad, model UV-VIS Double PC, United States of America). Each sample was analyzed in triplicate, and the absorbance values were used to calculate the average TPC, expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

# 3.2.2 Total Flavonoid Content

The total flavonoid content (TFC) of Torch ginger extracts was assessed using a colorimetric method described by Settharaksa et al. (2014). A 25μL sample of the Torch ginger extract (1 mg/mL) was combined with 125 µL of distilled water, followed by the addition of 25 μL of 5% sodium nitrate (NaNO<sub>3</sub>), and the mixture was incubated at room temperature for 6 minutes. Next, 15 uL of a 10% aluminum chloride (AlCl<sub>3</sub>) solution was added, and the mixture was left to incubate for another 5 minutes, after which 50 μL of 1 M sodium hydroxide (NaOH) was added. After 10 minutes, the absorbance at 510 nm was recorded using a microplate reader, and the TFC was determined using a standard curve derived from catechin concentrations (0-300 µg/mL). The results were reported as milligrams of catechin equivalent per gram of extract (mg catechin/g extract), calculated from the average of three replicates.

# 3.3 Biological Studies of Torch Ginger Inflorescence Extracts

#### 3.3.1 Antioxidant Activity Assays

1) DPPH Free Radical Scavenging Assay

The antioxidant activity of Torch ginger inflorescence extract was assessed using the DPPH assay, following the procedure described below. The sample and standard solutions were prepared at nine different concentrations (0.78 to 200 µg/mL) using a two-fold dilution method. Next, 100 µL of the sample solution and 100  $\mu$ L of DPPH solution (6 × 10<sup>-5</sup> M in ethanol) were added to a 96-well microplate, and the reaction was conducted in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a microplate reader. Each sample was tested in triplicate. The percentage of free radical inhibition (% inhibition) was calculated using Equation 1. A graph was plotted to show the relationship between the percentage of free radical inhibition and the concentration of the Torch ginger sample. The IC<sub>50</sub>

value was determined as the concentration of the Torch ginger extract required to inhibit 50% of DPPH radicals (Settharaksa et al., 2014).

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

# 2) ABTS Decolorization Assay

The antioxidant activity test was conducted using a modified method based on Sinsuebpol et al. (2023). The ABTS solution was prepared at a concentration of 7 mmol and mixed with 2.5 mmol potassium persulfate in a 2:1 ratio, then incubated at room temperature for 10 hours before being diluted and adjusted to an absorbance of  $0.700 \pm 0.02$  at 734 nm. Samples were prepared at concentrations between 25 and 500 μg/mL, and a 10 μL aliquot of each sample was mixed with 190 µL of the ABTS solution. The mixture was then incubated in the dark at room temperature for 5 minutes before the absorbance was measured at 734 nm using a microplate reader. Trolox was used as a standard reference compound. The percentage of ABTS radical inhibition (% ABTS inhibition) was determined using Equation 1. The radical scavenging activity was then plotted against concentration to calculate the IC<sub>50</sub> value. The experiment was performed in triplicate.

# 3.3.2 Anti-inflammatory Study

# 1) Inhibition of Nitric Oxide Assay

RAW264.7 cells ( $1 \times 10^5$  cells) in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin were seeded into each well of a 96-well plate. The cells were then incubated at  $37^{\circ}$ C in a 5% CO<sub>2</sub> atmosphere for 1 hour. The medium was then aspirated, and 1 µg/mL lipopolysaccharide (LPS) in RPMI-1640 medium was added to each well (100 µL for control and sample groups). RPMI-1640 medium alone was added to the blank well. LPS was used to stimulate macrophages to secrete inflammatory mediators.

Torch ginger inflorescence extract, at concentrations ranging from 1 to 100  $\mu$ g/mL, was added to the sample wells (100  $\mu$ L per well), while RPMI-1640 medium was added to the control wells. The plate was incubated at 37°C for 24 hours, after which 100  $\mu$ L of the culture medium from each well was transferred to a new 96-well plate and mixed with 100  $\mu$ L of Griess reagent. The absorbance was then measured at 570 nm using a microplate reader. Indomethacin was used as a standard reference in

the anti-inflammatory assay. The percentage of NO inhibition (% NO inhibition) was calculated using the following Equation 1. A graph was plotted to determine the relationship between % NO inhibition and sample concentration. The IC<sub>50</sub> value was calculated as the concentration of the sample extract required to inhibit NO production by 50% (Sudsai et al., 2014).

# 2) MTT Assay

RAW264.7 mouse macrophage cells were seeded into 96-well plates at a density of  $1 \times 10^5$  cells per well, treated with the test sample, and incubated in a 5% CO<sub>2</sub> environment for 24 hours. After incubation, 10  $\mu$ L of MTT solution (0.1 mg/mL) was added to each well, followed by an additional 2-hour incubation at 37°C. The culture medium was discarded, and the formazan crystals were dissolved in 100  $\mu$ L of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated using the following Equation 2. A sample was considered cytotoxic if cell viability was less than 80% compared to the control group (Sudsai et al., 2014).

% Cell survival = 
$$\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$
 (2)

# 3.3.3 Extracellular and Intracellular Melanin Content 1) Cell Viability Assay

Murine melanoma B16F10 cells were seeded into a 96-well plate at a density of  $1\times10^4$  cells per well. The cells were cultured at 37 °C in a 5% CO2 atmosphere for 24 hours. Subsequently, various concentrations of Torch ginger inflorescence extract and kojic acid (3.12-200  $\mu g/mL)$  were added, and the cells were incubated for an additional 48 hours. After incubation, cell viability was assessed using the MTT assay according to the standardized protocol. Cell viability was calculated using Equation 2.

#### 2) Extracellular Melanin Content

Extracellular and intracellular melanin produced by B16F10 cells was measured following the method of Junlatat et al. (2018). B16F10 cells were seeded into a 24-well plate at a density of  $1\times10^5$  cells per well. The cells were cultured at 37 °C in a 5% CO2 atmosphere for 24 hours. Subsequently, Torch ginger inflorescence extract and kojic acid, each at a concentration of 100  $\mu$ g/mL, were added. The control group received DMEM without plant extracts or kojic acid. The plates were then incubated for 48 hours. After incubation, the culture medium was carefully

transferred to a 96-well plate without disturbing the cells remaining in the 24-well plate. This step was performed to measure extracellular melanin. The absorbance of the transferred medium, which contained extracellular melanin, was measured at a wavelength of 405 nm using a microplate reader.

# 3) Intracellular Melanin Content

For the intracellular melanin assay, after incubating the cells with Torch ginger inflorescence extract or kojic acid for 48 hours, the cells were washed with PBS and detached from the 24-well plate using trypsin for 5 minutes. The harvested cells were then lysed with 800  $\mu$ L of 1 N NaOH containing 10% DMSO and incubated at 80 °C for 1 hour. After incubation, the lysates were centrifuged at 14,000 rpm for 15 minutes to separate the supernatant. Next, 100  $\mu$ L of the supernatant was transferred to a 96-well plate, and the absorbance was measured at 405 nm using a microplate reader (Junlatat et al., 2018).

#### 3.3.4 Anti-tyrosinase Activity

Tyrosinase activity was measured modifying the method of Sinsuebpol et al. (2023), which is based on the oxidation rate of L-tyrosine. The reaction mixture in each sample well consisted of the following: 40 µL of tyrosinase solution (200 units/mL), 80 µL of phosphate buffer (pH 6.8), 40 µL of Torch ginger inflorescence extract at various concentrations, and 40 µL of 10 mM L-tyrosine. These components were added to a 96-well plate and mixed thoroughly. The plate was incubated at room temperature for 30 minutes. The absorbance was then measured at 490 nm using a microplate reader. Kojic acid at a concentration of 100 µg/mL was used as a positive control for comparison with the sample group. Tyrosinase enzyme inhibition was calculated using Equation 1.

#### 3.4 Statistical Analysis

All data were presented as mean  $\pm$  standard error of the mean (SEM) based on three independent determinations. Statistical analysis was performed using one-way ANOVA, followed by Duncan's multiple range test in SPSS software. A *p*-value < 0.05 was considered statistically significant.

#### 4. Results and Discussion

#### 4.1 Plant Extraction

Different color varieties of Torch ginger inflorescence were extracted using the MAE method with three concentrations of ethanol (100%, 80%, and 50%). After extraction, the solvents were evaporated using a steam bath. A total of nine Torch ginger samples were obtained, as shown in Figure 1. The results of extraction of Torch ginger inflorescence using various ethanol concentrations showed that the crude extracts were a dark red viscous liquid (Figure 1). The extract yields of Torch ginger samples ranged from 1.4 to 3.3 g. It was found that the white Torch ginger inflorescence extract obtained with 80% ethanol had the highest yield, measuring 3.3 g, with a product percentage of 16.9%, as shown in Table 1. Research on the antioxidant and antibacterial properties of Torch ginger leaves and inflorescences has reported extract yields ranging from 4.1% to 6.0% (Chan et al., 2011b). The present study found that the MAE method resulted in a higher product yield compared to the aforementioned research. Extraction of active compounds from the Torch ginger inflorescence using 50% ethanol yielded the highest amount of crude extract by weight. However, biological activity testing showed that the crude extract obtained with 100% ethanol exhibited the strongest antioxidant and antiinflammatory activities, as well as the greatest reduction in melanin production through tyrosinase enzyme inhibition (Table 2 and Table 3).

The use of MAE can improve the efficiency of extracting active compounds from medicinal plants. Microwave energy is transmitted directly to the plant material through molecular interactions with electromagnetic fields, converting electromagnetic energy into heat. When water is used as the extraction solvent, it absorbs high levels of microwave energy but dissipates it slowly, leading to a superheating phenomenon. This occurs when water within the plant material heats up, causing the internal temperature to rise, which leads to cell rupture and the release of bioactive compounds from within the cells. Moreover, MAE is a convenient, fast, and energy-efficient method that reduces extraction time compared to conventional techniques. Additionally, it minimizes solvent usage, reduces costs, and prevents solvent residues from being released into the environment (Sánchez-Reinoso et al., 2020).



Figure 1 Dried inflorescence and the ethanolic extracts of red, pink and white Torch ginger inflorescence using the various ethanol concentrations

Table 1 Extraction yield, total phenolic and total flavonoid contents of Torch ginger inflorescence extracts

Sample	Extraction	% Yield	TPC	TFC (mg Catechin /g extract)	
Sample	yield (g)	(w/w)	(mg GAE/g extract)		
Red-varieties					
Absolute EtOH	2.4	11.8	$33.9\pm0.6$ a	$5.9\pm2.0$ a	
80% EtOH	2.3	11.3	$100.1 \pm 0.6 ^{\text{ f}}$	$38.8 \pm 3.9^{\text{ d}}$	
50% EtOH	2.6	13.2	$102.7 \pm 1.2 ^{\text{ f}}$	$56.7 \pm 2.4  ^{\mathrm{f}}$	
Pink-varieties					
Absolute EtOH	1.4	7.2	$38.3 \pm 1.2^{\ b}$	$5.9\pm2.5$ a	
80% EtOH	1.8	9.1	$96.4 \pm 0.5  ^{\mathrm{f}}$	$39.2\pm4.8^{\text{ d, e}}$	
50% EtOH	2.5	12.4	$60.7\pm0.8$ $^{\rm c}$	$25.4 \pm 3.2^{\ b}$	
White-varieties					
Absolute EtOH	2.6	13.2	$70.2 \pm 1.8$ d	$26.2 \pm 1.3$ b	
80% EtOH	3.3	16.9	$86.1\pm0.8$ $^{\mathrm{e}}$	$34.4\pm0.9^{c}$	
50% EtOH	3.1	15.5	85.2 ± 0.5 °	42.0 ± 1.4 °	

Each value represents the mean  $\pm$  SEM of three determinations

The values followed by the same letter in the column are not significantly different based on ANOVA followed by Duncan's multiple range test (p < 0.05)

Table 2 Antioxidant and anti-inflammatory activities of Torch ginger inflorescence extract

	Antioxida	nt activity	Anti-infl	Anti-inflammatory		
Sample	DPPH assay	ABTS assay	NO	MTT (LC <sub>50</sub> ; μg/mL)		
_	(IC <sub>50</sub> ; μg/mL)	(IC <sub>50</sub> ; μg/mL)	(IC <sub>50</sub> ; μg/mL)			
Red-varieties						
Absolute EtOH	$64.5 \pm 0.9^{e}$	$251.6 \pm 0.9^{e}$	$40.4\pm0.4^b$	> 100		
80% EtOH	$12.9 \pm 0.7^{b}$	50.3 ± 1.2 <sup>a</sup>	$46.9 \pm 0.9^{c}$	> 100		
50% EtOH	$12.5 \pm 0.3^{b}$	$48.8 \pm 2.3^{a}$	$52.2 \pm 0.6$ d	> 100		
Pink-varieties						
Absolute EtOH	$60.0\pm1.3^{e}$	$282.3 \pm 3.7^{e}$	$41.4\pm0.8^b$	> 100		
80% EtOH	$28.3\pm2.1^{\rm d}$	$133.0 \pm 4.1^{\circ}$	$47.5\pm0.5^{c}$	> 100		
50% EtOH	$44.5 \pm 1.4^{d}$	$209.2\pm2.8^{\rm d}$	$57.4 \pm 0.7^{\rm e}$	> 100		
White-varieties						
Absolute EtOH	29.6 ± 1.1°	$112.5 \pm 2.2^{c}$	$40.5 \pm 0.5^{b}$	> 100		
80% EtOH	$17.7 \pm 2.6^{b}$	$69.0 \pm 4.8^{b}$	54.1 ± 1.1 <sup>d</sup>	> 100		
50% EtOH	$17.1 \pm 1.2^{b}$	$66.7 \pm 3.7^{b}$	$47.2\pm0.7^c$	> 100		
Ascorbic acid	$1.6\pm0.3^{\rm a}$	-	-	-		
Quercetin	$0.4\pm0.0^{\mathrm{a}}$	-	-	-		
BHT	$15.8\pm1.4^{b}$	-	-	-		
Trolox	-	$49.8\pm0.5^a$				
Indomethacin	-	-	27.7 ± 0.7 a	≥ 100		

Each value represents the mean  $\pm$  SEM of three determinations

 $IC_{50}$  values followed by the same letter in the column are not significantly different based on ANOVA followed by Duncan's multiple range test (p < 0.05)

# 4.2 Phytochemical Analysis

In this study, the quantitative determination of two major classes of antioxidant compounds, TPC and TFC, was carried out. TPC was measured using the Folin-Ciocalteu colorimetric assay and expressed as mg GAE/g extract, while TFC was determined using the aluminum chloride method and reported as mg catechin/g extract. These assays provide the extract's phenolic and flavonoid profiles. Therefore, TPC and TFC of different color varieties of Torch ginger inflorescence extracts were examined. The TPC of Torch ginger inflorescence extracts was determined using gallic acid as a standard. The standard calibration curve followed the equation y = 0.0034x + 0.0185 (R<sup>2</sup> = 0.9946). The total phenolic content of Torch ginger samples ranged from 33.9 to 102.7 mg GAE/g extract. It was found that the red Torch ginger inflorescence extract had the highest phenolic content. The results indicated that the red inflorescence extracted with 50% ethanol had the highest total phenolic content, measuring 102.7 mg GAE/g extract, followed by other samples. The flavonoid content was analyzed using catechin as the standard, with the calibration curve equation y = 0.0021x + 0.0047 (R<sup>2</sup> = 0.9961). The flavonoid content in Torch ginger inflorescence extracts ranged from 5.9 to 56.7 mg catechin/g extract. The results

indicated that the red inflorescence extracted with 50% ethanol had the highest flavonoid content, measuring 56.7 mg catechin/g extract, as shown in Table 1.

Phytochemicals found in plants belong to various groups, such as phenolic acids, terpenoids, alkaloids, and flavonoids. These compounds have distinct chemical properties, including differences in polarity, molecular weight, and volatility. Various methods and techniques are used to extract active compounds from plants. Solvent extraction using mixtures such as water, acetone, or alcohol can influence both the quantity and quality of the extracted compounds. This is because a mixture of a highly polar solvent, such as water, and a moderately polar solvent, such as alcohol or acetone, can extract a higher amount of crude extract. This finding is consistent with the results reported by Poonpaiboonpipat et al. (2011), who found that the crude extract from jasmine leaves using 50% ethanol mixed with distilled water yielded a greater amount of extract compared to using ethanol or water alone. However, when evaluating the effect of crude extracts on the inhibition of biological activities, it was found that the 100% ethanolic extract exhibited the strongest inhibitory effects. This suggests that ethanol alone is more effective than an ethanol-water mixture in extracting

active compounds from Torch ginger inflorescences. Most of the phytochemicals found in Torch ginger inflorescences are water-insoluble compounds, such as phenolic acids, fatty acids, flavonoids, tannins, saponins, and terpenoids (Maimulyanti & Prihadi, 2015). Phytochemical study of Torch ginger extracts confirmed the presence of phenols, flavonoids, terpenoids, saponins, tannins, and carbohydrates. Additionally, phenolic compounds, flavonoids, anthocyanins, and tannins were detected in high amounts in the inflorescences. Several flavonoids have been identified in Torch ginger leaves, stems, rhizomes, and inflorescences, including kaempferol, apigenin, luteolin, quercetin, kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3glucoside, and quercetin 3-rhamnoside. Additionally, the inflorescences contain other phenolic compounds, including caffeoylquinic and chlorogenic acid, as well as flavonoids like quercitrin, isoquercitrin, and catechin (Juwita et al., 2018; Chan et al., 2011b).

# 4.3 Biological Studies of Torch Ginger Inflorescence Extracts

# 4.3.1 Antioxidant Activity

The antioxidant activity of Torch ginger inflorescence extracts obtained through MAE was assessed using DPPH and ABTS assays to determine their antioxidant potential. The DPPH antioxidant activity test was conducted on three colors of Torch ginger inflorescence extracted with 100%, 80%, and 50% ethanol. The results showed that red inflorescences extracted with 50% ethanol exhibited the highest antioxidant activity, with an IC<sub>50</sub> value of 12.5 µg/mL. The standard antioxidants used for comparison were vitamin C (IC<sub>50</sub> = 1.6 µg/mL), quercetin (IC<sub>50</sub> = 0.4  $\mu$ g/mL), and BHT (IC<sub>50</sub> = 15.8 μg/mL), as shown in Table 2. When compared with standard antioxidants, including vitamin C, quercetin, and BHT, the red flower extract demonstrated lower antioxidant activity than vitamin C ( $IC_{50} = 1.6 \mu g/mL$ ) and quercetin (IC<sub>50</sub> =  $0.4 \mu g/mL$ ), but showed activity comparable to BHT (IC<sub>50</sub> = 15.8  $\mu$ g/mL).

The results of the ABTS radical inhibition study, based on the IC<sub>50</sub> values of the Torch ginger inflorescence extracts ranged from 48.8 to 282.3  $\mu g/mL$ . Consistent with the DPPH assay results, the crude extract of red Torch ginger inflorescence obtained with 50% ethanol had an IC<sub>50</sub> value of 48.8  $\mu g/mL$ , indicating that this extract exhibited the highest ABTS antioxidant activity. The standard antioxidant used for comparison was Trolox (IC<sub>50</sub> = 48.9  $\mu g/mL$ ), as shown in Table 2. The experimental

results demonstrated that antioxidant activity, as measured by DPPH and ABTS assays, was related to the total phenolic and flavonoid content. Previous studies have also reported that higher phenolic content corresponds to increased antioxidant activity (Sinsuebpol et al., 2023).

The findings from the antioxidant activity test, along with the analysis of phenolic and flavonoid contents, indicated that the red Torch ginger inflorescence extracted with 50% ethanol provided the best results, yielding 102.7 mg GAE/g of TPC and 56.7 mg catechin/g of total TFC, respectively. These findings align with previous research on the optimal solvent system for extracting phenolic compounds, flavonoids, and antioxidant activity from marigold leaf extract. Their study examined 12 different solvent systems, identifying that 40-60% ethanol provided the highest phenolic, flavonoid, and antioxidant activity (Ćetković et al., 2004). The quantification of phenolic compounds and flavonoids revealed that red Torch ginger inflorescence contained the highest levels, followed by pink and white flowers, respectively. This trend may be due to the presence of red pigments, especially anthocyanins, which are part of the phenolic compound group. Studies on the antioxidant capacity of red and white edible flowers have similarly shown that red flowers contain higher levels of phenolic compounds and demonstrate stronger antioxidant activity than white flowers. Additionally, red flowers have been found to possess greater concentrations of phenolic compounds and flavonoids compared to other flower color groups (Prabawati et al., 2021).

# 4.3.2 Anti-inflammatory Activity

The anti-inflammatory activity test, based on the inhibition of nitric oxide (NO) secretion in RAW264.7 cells, macrophage measures the concentration of nitrite in the cell culture medium as an index of NO production. Nitrite is a stable product of NO oxidation, making it a reliable indicator (Sudsai et al., 2014). From the results of the anti-inflammatory activity test using the NO secretion inhibition method, extracts from three Torch ginger inflorescence varieties red, pink, and white color were evaluated after extraction with 100%, 80%, and 50% ethanol. The results indicated that red Torch ginger inflorescences extracted with 100% ethanol exhibited the highest anti-inflammatory activity, with an IC<sub>50</sub> value of 40.4 µg/mL. Meanwhile, indomethacin, used as the standard reference compound, had an IC<sub>50</sub> value of 27.7 µg/mL, as shown in Table 2. However, when

compared to the standard anti-inflammatory agent indomethacin, the extract demonstrated lower activity. Research has been conducted on the anti-NO production of Torch ginger inflorescence extract; however, studies on the anti-inflammatory activity of Torch ginger remain limited. The potent anti-inflammatory activity of Torch ginger was demonstrated through the inhibition of NO production by its rhizome extract, showing efficacy comparable to indomethacin (Srey et al., 2014). However, extracts from all colors of Torch ginger inflorescences exhibited low anti-inflammatory activity in inhibiting IL-1 and TNF-α, which are inflammatory cytokines (Sinsuebpol et al., 2023).

The cell survival test was performed using the MTT assay, which measures mitochondrial dehydrogenase enzyme activity in viable cells. In this assay, MTT is reduced by mitochondrial enzymes to form purple formazan crystals, and the amount of formazan produced is directly proportional to the number of viable cells (Sudsai et al., 2014). The cell survival test showed that the microwave extract of Torch ginger inflorescence did not affect cell viability, as cell death remained below 20% at the highest tested concentration (100 µg/mL) indicating that the extract was not toxic to cells.

In inflammation, NO and PGE<sub>2</sub> are produced in high amounts in LPS-stimulated macrophages, leading to increased iNOS and COX-2 enzyme activity. These mediators play a crucial role in the inflammatory process. Molecular studies have shown that the regulation of iNOS and COX-2 gene expression occurs at the transcriptional level, controlled by nuclear factor-κB (NF-κB). Structural analysis of the 5'-flanking region of these genes has identified a cis-acting NF-κB element. The activation of IkB kinase (IKK) triggers the phosphorylation and degradation of the inhibitor kB (IkB) protein, leading to the release and nuclear translocation of NF-κB. Once inside the nucleus, NF-kB binds to the cis-acting NF-κB element on the iNOS and COX-2 genes, activating them and promoting inflammation (Sudsai et al., 2014). The results of this study showed that extracts from the inflorescences of Torch ginger inhibited the production of NO and may also inhibit the expression of iNOS, which is a key enzyme involved in the formation of these inflammatory mediators. Thus, the observed reduction in NO levels following treatment with Torch ginger extracts suggests that the extracts may inhibit iNOS and COX-2 gene expression, thereby suppressing the inflammatory process.

4.3.3 Extracellular and Intracellular Melanin Content

In this study, crude extracts of Torch ginger inflorescence, prepared using various concentrations of ethanol, were tested on B16F10 cells-melanocytes derived from murine melanoma. B16F10 cells are commonly used as an in vitro model to evaluate melanin inhibitors or skin-lightening agents (Lee et al., 2019). The first step is to assess cellular toxicity to determine whether the tested extract is toxic to B16F10 cells and, if so, at what concentration. A nontoxic concentration of the Torch ginger inflorescence extract is then selected to evaluate its melanininhibiting effect on B16F10 cells. To evaluate cellular toxicity, B16F10 cells were treated with Torch ginger inflorescence extract at concentrations ranging from 3.12 to 200 µg/mL and incubated for 48 hours. Cell viability was assessed using the MTT method. The results showed that all concentrations of the extract significantly reduced cell viability compared to the control. However, B16F10 cells incubated with Torch ginger inflorescence extract at a concentration of 100 μg/mL showed a cell viability percentage above 80%, except for the 50% and 80% ethanolic extracts of the red variety (Table 3). Therefore, a concentration of 100 μg/mL was selected to evaluate the extract's effect on melanin reduction and tyrosinase enzyme inhibition.

Torch ginger inflorescence extract at a concentration of 100 µg/mL was incubated with B16F10 cells for 48 hours and compared to kojic acid, a standard substance used to inhibit melanin production, at the same concentration. The results showed that Torch ginger inflorescence extract at a concentration of 100 µg/mL significantly inhibited melanin production compared to the control group, with an inhibition rate of approximately 8.9% -33.3%. In contrast, Kojic acid at concentrations of 100 μg/mL showed a significantly different inhibition effect on extracellular melanin production from all experimental groups (Table 3). The results of intracellular melanin content in B16F10 melanoma cells treated with Torch ginger inflorescence extract for 48 hours showed that the extract, at a concentration of 100 µg/mL, inhibited melanin production by approximately 10.5% - 38.0%, which was significantly different from the control group. Kojic acid, at the same concentration, demonstrated a greater inhibitory effect on melanin production, with a reduction of 46.1% (Table 3).

Melanin functions to filter ultraviolet (UV) light from sunlight and helps scatter light, particularly short-wavelength light such as blue-violet light, away

from the skin. However, excessive melanin production can lead to skin pigmentation disorders (Pillaiyar et al., 2017). Factors that affect melanin production include UV radiation and genetics. UV rays influence melanin production in two ways: (1) they directly stimulate melanocytes, enhancing the activity of the tyrosinase enzyme, which leads to increased melanin production. Greater melanin levels result in darker skin pigmentation. (2) UV rays stimulate keratinocytes, triggering a paracrine regulation process in which keratinocytes release various substances, such as prostaglandin E2 (PGE2) and α-melanocyte-stimulating hormone ( $\alpha$ -MSH).  $\alpha$ -MSH is a hormone secreted by the anterior pituitary gland and also produced by melanocytes. This hormone stimulates melanin production through cyclic AMP (cAMP), which activates protein kinase A and accelerates the phosphorylation process. This, in turn, activates the microphthalmia-associated transcription factor (MITF gene), a key regulatory gene that controls melanocyte function, promoting cell proliferation, melanin production, and regulation of the melanocyte life cycle. It also regulates the DNA replication of enzymes involved in the melanin production process, such as tyrosinase, TRP-1, and TRP-2. Additionally, melanocytes act as phagocytic cells that respond to

skin inflammation by stimulating the secretion of cytokines like interleukin-1, which activates MSH receptors and increases melanin production. Similarly, adrenocorticotropic hormone (ACTH) stimulates the release of nitric oxide, which enhances the activity of  $\alpha$ -MSH (D'Mello et al., 2016; Slominski et al., 2012). As mentioned earlier, tyrosinase is the key enzyme in melanin production; therefore, the effect of Torch ginger inflorescence extracts on inhibiting tyrosinase activity was evaluated in vitro.

# 4.3.4 Anti-tyrosinase Activity

The key to the melanin biosynthesis pathway lies in the enzyme tyrosinase, as it catalyzes the rate-limiting step of melanin production. Therefore, it is considered the rate-limiting enzyme that regulates the overall biochemical process of melanin synthesis in melanocytes (Panzella, & Napolitano, 2019). This enzyme catalyzes the hydroxylation of tyrosine to DOPA and the oxidation of DOPA to DOPAchrome. Therefore, an inhibitor of tyrosinase activity can prevent excessive melanin accumulation in the skin (El-Nashar et al., 2021). For this reason, identifying plant extracts that can inhibit tyrosinase activity is essential for their use as ingredients in cosmetics and skin-lightening products (Lee et al., 2019).

Table 3 Melanin content and anti-tyrosinase activity of Torch ginger inflorescence extract in B16F10 cells

Sample	Viability		Melanin content (% relative to control)		Anti- tyrosinase
	LC <sub>50</sub> (μg/mL)	% relative to control	Extracellular	Intracellular	(% inhibition)
Control	-	$100\pm0.0~^{\rm e}$	$100\pm0.0~^{h}$	$100\pm0.0~^{\rm g}$	-
Red-varieties					
Absolute EtOH	≥ 200	$85.2 \pm 1.4^{b}$	$81.1\pm2.2~^{\rm f}$	$62.0\pm1.8^{\text{ c}}$	$31.3\pm1.4~^{\rm f}$
80% EtOH	≥ 100	$74.6 \pm 0.7^{*,a}$	$62.8 \pm 1.6$ b, c	$58.3 \pm 2.0^{b,c}$	$14.3\pm1.0^{c}$
50% EtOH	$112.0\pm2.6$	$71.6 \pm 1.8^{*, a}$	$61.3 \pm 1.3$ b	$54.9\pm0.7^{\mathrm{\;a,\;b}}$	$8.5\pm0.3$ b
Pink-varieties					
Absolute EtOH	≥ 200	$95.5 \pm 0.9$ d	$91.1 \pm 1.9$ g	$89.5 \pm 1.0^{\text{ f}}$	$12.7 \pm 0.5$ °
80% EtOH	> 200	$83.3 \pm 1.6$ b	$81.1 \pm 1.1 ^{\text{f}}$	$72.9\pm1.2^{\rm \ d}$	$19.1 \pm 1.0^{d}$
50% EtOH	> 200	$89.9 \pm 1.4^{\rm c}$	$89.2\pm1.5~^{\rm g}$	$84.3 \pm 2.1$ e	$5.5\pm0.6^{\rm \ a}$
White-varieties					
Absolute EtOH	≥ 200	$94.9\pm1.3~^{\rm d}$	$75.4 \pm 1.8$ $^{\rm e}$	$62.4\pm1.9^{c}$	$32.6\pm1.4~^{\rm f}$
80% EtOH	> 200	$89.1\pm0.7$ °	$66.6 \pm 1.5^{\text{ c, d}}$	$82.2\pm2.4$ e	$23.1\pm1.0^{\text{e}}$
50% EtOH	> 200	$84.4\pm0.7^{b}$	$70.6\pm3.2^{\text{ d, e}}$	$90.9 \pm 2.1  ^{\mathrm{f}}$	$7.0\pm2.3$ a, b
Kojic acid	> 200	$91.9 \pm 1.8^{c,d}$	$47.2\pm0.6$ a	$52.9\pm1.4^{a}$	$70.1\pm0.2~^{\rm g}$

Each value represents the mean  $\pm$  SEM of three determinations

The values followed by the same letter in the column are not significantly different based on ANOVA followed by Duncan's multiple range test (p < 0.05)

<sup>\*</sup>Cytotoxic effect was observed

The anti-tyrosinase activity of Torch ginger inflorescence extracts was tested at a concentration of 100 µg/mL using L-tyrosine as the substrate. The extracts showed relatively low tyrosinase inhibition, ranging from approximately 5.5% to 32%, and were less effective than kojic acid (Table 3). Among the samples, the red and white Torch ginger inflorescences extracted with 100% ethanol exhibited the highest inhibitory activity at 31% and 32%, respectively. In comparison, kojic acid, used as the standard reference compound, showed 70% inhibition at the same concentration (Table 3). Chang (2009) proposed that phytochemicals inhibit tyrosinase activity through a mechanism known as competitive inhibition. These compounds compete with the substrate for binding to the active site of the tyrosinase enzyme. Phytochemicals that act as competitive inhibitors may function as copper chelators, nonmetabolizable analogs, or derivatives of the natural substrate. A commonly discussed mechanism involves certain phytochemicals acting as chelating agents, capable of binding to positively charged copper ions (Cu<sup>2+</sup>). This interaction allows them to bind to free tyrosinase enzymes, which contain copper at their active sites. Therefore, when chelation occurs between phytochemical molecules and copper ions, the enzyme becomes inactive or exhibits reduced activity, as fewer substrate molecules can bind to the active site of tyrosinase. This leads to a decreased rate of melanin synthesis (Panzella & Napolitano, 2019).

In this study, a qualitative phytochemical analysis revealed that Torch ginger inflorescence extracts contain phenolic compounds and flavonoids. These findings are consistent with previous research that used high-performance liquid chromatography (HPLC) to analyze the phytochemical content of Torch ginger inflorescence extracts, showing that the ethanolic extract is rich in phenolic and flavonoid compounds, which are prominent constituents (Sinsuebpol et al., 2023). In addition, analysis using coupled chromatography with spectrometry (LC-MS/MS) revealed that the Torch ginger inflorescence extract contains compounds such as amino acids, carboxylic acids, and phenolic acids, as well as flavonoids including catechin, isoquercitrin, kaempferol-7-O-glucoside, among (Mutmainah et al., 2024). It was also found that phenolic compounds contribute to the inhibition of melanin production. For example, gallic acid acts as a substrate analog for tyrosinase, being oxidized in place of tyrosine. Due to its structure, gallic acid can form an ester bond with the enzyme, thereby

inhibiting its activity. Similarly, ellagic acid inhibits tyrosinase by binding to copper ions, reducing the enzyme's activity. Additionally, ellagic acid suppresses melanocyte proliferation and melanin synthesis (Draelos et al., 2010). Therefore, the tyrosinase inhibitory effect of Torch ginger inflorescence extracts observed in this study may be attributed to the flavonoids present, which may act as competitive inhibitors. These flavonoids can bind to copper ions-key components of the active site of the tyrosinase enzyme-according to the competitive inhibition mechanism described above.

#### 5. Conclusion

This study investigated the biological activities of Torch ginger inflorescence extracts prepared by MAE using three different inflorescence colors and three ethanol concentrations. The results revealed that the red Torch ginger inflorescence yielded extracts with significantly higher TPC and TFC compared to the white inflorescence, which likely contributes to its stronger antioxidant and anti-inflammatory activities. In contrast, white inflorescence extracts exhibited comparable bioactivities, with slightly lower IC50 values in some assays, while yielding a higher overall extraction mass. This trade-off between extraction efficiency and phenolic and flavonoid richness suggests that red inflorescences may be preferable when aiming for maximal bioactivity, whereas white inflorescences could offer advantages for large-scale extraction due to their greater mass recovery. Based on these findings, the researchers suggest that red Torch ginger inflorescence extracts have biological potential for use in cosmetic applications.

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#### 7. CRediT Statement

**Watchara Chongsa**: Laboratory experiments, data curation, writing – review & editing.

**Tun Chusut**: Laboratory experiments, statistical analysis.

**Teeratad Sudsai**: Conceptualization, methodology, laboratory experiments, writing – original draft – review & editing, supervision, funding acquisition.

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