TRENDS IN SCIENCES 2026; 23(1): 11354
https://doi.org/10.48048/tis.2026.11354 RESEARCH ARTICLE

Impact of Gamma Ray and X-ray Irradiation on the Microbial Load and Cosmeceutical Properties of Thanaka Powder

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Received: 22 July 2025, Revised: 14 August 2025, Accepted: 21 August 2025, Published: 30 October 2025

Abstract

Thanaka (*Hesperethusa crenulata* Roem) is a traditional herbal powder widely used in cosmetic formulations for its skin-protective properties. However, microbial contamination during processing poses a safety concern. This study evaluated the effects of gamma ray and X-ray irradiation (5 - 20 kGy) on the microbial load and cosmeceutical properties of Thanaka powder. Non-irradiated Thanaka had TVC of 3.0×10^3 and TYM of 2.0×10^2 CFU/g. Irradiation ≥ 5 kGy reduced both to undetectable levels, meeting Thai FDA standards. Minor color changes like dose-dependent darkening and a green-to-red shift occurred but did not affect the powder's cosmetic quality. Key bioactive compounds, including arbutin (2.06 - 2.16 mg/mL) and marmesin (0.12 - 0.14 mg/mL), as well as tyrosinase inhibitory activity (8.10% - 8.85%), sun protection factor (SPF, 6.17 - 6.89), total phenolic content and antioxidant activity (DPPH, FRAP), remained stable post-irradiation. These findings indicate that ionizing radiation is an effective method for enhancing the microbiological quality of Thanaka powder without compromising its functional or aesthetic qualities, with X-ray irradiation offering a practical alternative to gamma ray.

Keywords: Gamma ray, X-ray, Irradiation, Microbiological quality, Cosmeceutical properties, Arbutin, Marmasin

Introduction

Thanaka, derived from the bark of *Hesperethusa crenulata* Roem (syn. *Naringi crenulata*), is a traditional herbal powder widely used in Southeast Asia, particularly in Myanmar and Thailand, for cosmetic and dermatological purposes. When applied to the skin as a paste, it provides a cooling sensation, UV protection, anti-inflammatory effects, and skin-lightening properties. The benefits of Thanaka can be largely attributed to its high phenolic content. Among the key bioactive compounds in Thanaka are arbutin, a strong tyrosinase inhibitor that reduces melanin production and helps address hyperpigmentation and marmesin, a furocoumarin that absorbs ultraviolet light through its chromophore structures [1]. These compounds not only provide skincare benefits but also contribute to the antioxidant and anti-inflammatory properties of Thanaka while exhibiting minimal cytotoxicity. This property makes it a valuable ingredient in cosmetic formulations. The pale yellow to beige color of Thanaka signals authenticity and influences consumer preference. However, the color of herbal materials is often affected by environmental factors and processing conditions, which can lead to undesirable changes in appearance and decreased consumer acceptance. The global herbal cosmetics market is projected to grow by 9.9% annually from 2024 onwards, driven by demand for plant-based alternatives to synthetic chemicals [2], the stability and safety of herbal products like Thanaka are becoming increasingly important for regulatory approval and market success.

Microbial contamination during harvesting, processing, and storage threatens the safety and quality

of Thanaka powder, despite its beneficial properties. This raises safety concerns, particularly for cosmetic applications where microbial contamination is tightly regulated. The Thai Food and Drug Administration (Thai FDA) sets strict microbial limits, including total viable counts of no more than 1.0×10^3 CFU/g and total yeast and mold counts of no more than 1.0×10^2 CFU/g. Pathogens such as *Salmonella* spp., *Escherichia coli*, and *Bacillus cereus* are not permitted [3]. Surveys of Thanaka products sold in Myanmar and near the Thai Myanmar border have shown microbial contamination levels that exceed these limits [4]. Conventional decontamination methods often compromise these qualities or have practical drawbacks. Therefore, ionizing radiation, a non-thermal sterilization technique known for effective microbial control with minimal impact on bioactive compounds, offers a promising alternative.

Ionizing radiation offers a potential non-thermal sterilization approach to overcome these limitations. Irradiation has also been reported to decontaminate dried herbal and cosmetic materials without significantly altering their physical characteristics [5]. In certain instances, the irradiation also enhances the appearance of herbal materials. For example, it enhanced the brightness and uniformity of *Curcuma aromatica* extracts, shifting their color from dull to vibrant yellow while preserving curcuminoid content [6]. Owing to their superior penetration depth, gamma rays and X-rays are well suited for the treatment of dense or bulk materials, whereas electron beams are more appropriate for surface sterilization [7]. Gamma irradiation using cobalt-60 is a well-established technique, while X-rays generated via high-energy electron conversion present a promising alternative, offering on-demand operation and eliminating the need for radioactive sources [8]. Most previous studies have employed gamma radiation to reduce microbial contamination and assess its effects on chemical properties [9], whereas the impact of X-ray irradiation on the cosmeceutical properties of Thanaka powder has not been investigated. Moreover, comparative studies evaluating gamma and X-ray irradiation in herbal powders remain scarce. This study addresses these knowledge gaps, providing comprehensive data to support the potential transition from gamma to X-ray irradiation technology.

This study aims to evaluate the effects of gamma and X-ray irradiation at doses ranging from 5 to 20 kGy on the microbial safety and stability of bioactive compounds in Thanaka powder. The dose range used complies with Thailand's domestic regulation (<10 kGy) and the U.S. FDA export limit (<30 kGy) for herbal products [10]. We hypothesize that X-ray irradiation can achieve microbial reduction comparable to gamma rays while better preserving bioactives and visual quality. By identifying optimal irradiation conditions, this research aims to support the development of safer, longer-lasting Thanaka-based cosmetics that comply with regulatory standards and consumer expectations, ultimately enhancing product quality and competitiveness in the growing herbal cosmetics market.

Materials and methods

Sample preparation and irradiation

Commercially dried Thanaka powder *(Hesperethusa crenulata* Roem) was purchased from a local supermarket in Bangkok, Thailand and packaged in aluminum foil bags to prevent moisture and light exposure during storage. The samples were subjected to ionizing radiation at 2 facilities of the Thailand Institute of Nuclear Technology (Public organization). Gamma irradiation was performed using a cobalt-60 chamber (BRIT, India) in Nakhon Nayok Province, while X-ray irradiation was carried out with a high-energy electron accelerator equipped with an X-ray converter (MB5-50, Mevex Corporation Ltd., Canada) in Pathum Thani Province. The powders were irradiated at doses of 5, 10, 15 and 20 kGy. Dose verification was conducted using amber Perspex polymethylmethacrylate (PMMA) dosimeters for gamma irradiation and alanine dosimeters for X-ray irradiation. Each treatment was performed in triplicate. All irradiated and non-irradiated samples were stored at ambient temperature in the dark until further analysis.

Sample extraction

Crude extracts were prepared for phytochemical and antioxidant analyses. For all assays except marmesin quantification, 10 mg of Thanaka powder was extracted with 10 mL of 60% ethanol by ultrasonic-assisted extraction for 60 min. For marmesin analysis, the powder was extracted using a 1:1 (v/v)

chloroform:methanol mixture under the same ultrasonic conditions. Extracts were centrifuged at 8,000 rpm at 4 °C for 10 min. The supernatants were filtered through 0.45 µm syringe filters and stored in amber vials at –20 °C until analysis.

Microbiological analysis

Total viable count (TVC) and total yeast and mold count (TYM) were determined following the FDA Bacteriological Analytical Manual [11,12]. Bacillus cereus was enumerated using the ISO 7932:2004 standard protocol [13]. All microbial analyses were conducted using 25 g of sample suspended in 225 mL of sterile normal saline solution. After thorough mixing, serial 10-fold dilutions were prepared, and 0.1 mL of each dilution was spread onto appropriate agar media and results were expressed as CFU/g.

Color measurement

The color of each sample (non-irradiated and irradiated) was measured using a Chroma Meter (CR-300, Minolta, Japan). Measurements were based on the Hunter Lab color system, with lightness (L*), redness (a*), and yellowness (b*) values recorded. The instrument was calibrated using a white reference tile prior to measurement.

Assessment of arbutin and marmesin content

Arbutin and marmesin content analyses were carried out using High-Performance Liquid Chromatography (HPLC) equipment (Waters e2695 Alliance, USA) with a UV detector. The separation was carried out using a C18 column (Sunfire 5 µm, 150×4.6 mm^2). For arbutin content analysis, a linear gradient elution of acetonitrile and 3% acetic acid was applied, starting at a 10:90 (v/v) ratio at 0 min, increasing to 55:45 at 27 min, and held constant thereafter [14]. Arbutin was detected at 280 nm. Marmesin content was analyzed using an isocratic mobile phase of 50% methanol (flow rate 1.5 ml/min) run at ambient temperature for 15 min [15]. Standard curves were constructed using commercial standards (Sigma-Aldrich, USA), and all calibration curves had R^2 values > 0.995.

Tyrosinase inhibition assay

Tyrosinase inhibition activity was assessed using a modified DOPA-chrome method [16]. Mushroom tyrosinase (100 U/mL) and L-DOPA (1 mg/mL) were used as enzyme and substrate, respectively. Reaction tubes were prepared as follows:

Tube A: 0.35 mL tyrosinase + 0.35 mL phosphate buffer (0.02 M, pH 6.8) + 0.35 mL sample solvent (blank control)

Tube B: 1.05 mL phosphate buffer (blank reference)

Tube C: 0.35 mL tyrosinase + 0.35 mL phosphate buffer + 0.35 mL sample extract (test)

Tube D: 0.70 mL phosphate buffer + 0.35 mL sample extract (sample blank)

Each tube received 0.35 mL of L-DOPA and was incubated at 37 °C for 20 min. Absorbance was measured at 450 nm. Kojic acid (0.1 mg/mL) was used as a positive control. The percentage of inhibition was calculated as:

$$\text{Tyrosinase inhibition (\%)} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100$$

where A, B, C, and D represent the absorbance values of tubes A, B, C, and D, respectively.

Sun protection factor (SPF)

The photoprotective activity of the crude extracts was evaluated in vitro by estimating the Sun Protection Factor (SPF) using a modified Mansur equation [17]. The absorbance of the extracts was measured using a UV-Vis spectrophotometer equipped with a 1 cm quartz cuvette across the wavelength range of 290 - 320 nm at 5 nm intervals. Each sample was tested at a concentration of 2 mg/mL (dry weight basis). The SPF value was calculated based on the absorbance data using the following formula:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)$$

where: CF is the correction factor (typically 10), EE(λ) is the erythemal effect spectrum, I(λ) is the solar intensity spectrum and Abs(λ) is the absorbance of the sample at each wavelength λ. According to standard

literature, the value of EE(λ) × I(λ) remained constant and was used to compute the final SPF.

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method, with slight modifications from Velioglu *et al.* [18]. Briefly, 0.10 mL of extract was mixed with 0.75 mL of 10 %(v/v) Folin-Ciocalteu reagent and allowed to stand at room temperature for 5 min. Then, 0.75 mL of 6 %(w/v) sodium carbonate solution was added. After incubation at room temperature for 90 min, absorbance was measured at 725 nm using a UV-Vis spectrophotometer. Results were expressed as milligrams of gallic acid equivalents per gram of crude extract (mg GAE/g), based on a gallic acid standard curve. The calibration curve was linear within the tested range, with the equation $y = 6.78x - 0.0132$ and a coefficient of determination (R^2) of 0.9956.

Determination of antioxidant properties by DPPH and FRAP assay

The free radical scavenging activity was evaluated using the DPPH assay, with slight modifications from Khattak *et al.* [19]. A 0.10 mL aliquot of extract was mixed with 0.90 mL of 0.2 mM DPPH solution in methanol, vortexed, and incubated in the dark at room temperature for 15 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant activity was expressed as milligrams of ascorbic acid equivalents per gram of crude extract (mg AAE/g), based on a standard calibration curve of ascorbic acid. The calibration curve followed the equation $y = -11.579x + 0.7824$ with a coefficient of determination (R^2) of 0.9962.

The ferric reducing antioxidant power (FRAP) assay was performed according to the modified method of Benzie and Strain [20]. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 (v/v/v) ratio. A 2.70 mL aliquot of the reagent was added to 0.30 mL of extract, and the mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 615 nm using a UV-Vis spectrophotometer. Antioxidant capacity was expressed as millimoles of Fe^{2+} equivalents per gram of crude extract (mM Fe^{2+}/g), based on a standard curve of $FeSO_4 \cdot 7H_2O$. The calibration curve followed the equation $y = 0.0021x - 0.0211$ with a coefficient of determination (R^2) of 0.9994.

Statistical analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using 1-way ANOVA in SPSS software (version 29.0; IBM Corp., Armonk, NY, USA; license code: e3131844ec9137676f12). Assumptions of normality and homogeneity of variances were verified prior to analysis. Differences between means were assessed using Duncan's multiple range test at a significance level of $p < 0.05$.

Results and discussion

Microbial decontamination

The microbial load of Thanaka powder before and after irradiation at doses of 5, 10, 15, and 20 kGy is summarized in **Table 1**. The non-irradiated sample showed a total viable count (TVC) of 3.0×10^3 CFU/g and a total yeast and mold count (TYC) of 2.0×10^2 CFU/g, both exceeding the Thai FDA cosmetic limits. *Bacillus cereus* was not detected in any sample, regardless of treatment. Gamma and X-ray, as forms of ionizing radiation, penetrate microbial cells by ionizing intracellular water molecules, generating reactive oxygen species (ROS). These ROS damage critical cellular components such as DNA, proteins, and lipids. The most critical damage is caused by double-strand breaks in DNA, which are regarded as the most lethal, resulting in cell death or loss of reproductive capacity [21]. These mechanisms align with previous studies on irradiated herbal products, which reported significant reductions in TVC and TYC. Pathogens including *Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica* Typhimurium were effectively eliminated at doses between 5 and 10 kGy [22].

In this study, both gamma and X-ray irradiation at a minimum dose of 5 kGy reduced microbial loads to below detectable levels (ND) for TVC and TYC, with consistent efficacy across all tested doses (5 - 20 kGy). Importantly, ionizing radiation can be used for decontamination without degrading functional material performance [23], making it particularly suitable for preserving the quality of herbal cosmetic powders.

These findings support the use of X-ray irradiation as a practical alternative to gamma rays for microbial decontamination in dried herbal powders, particularly for cosmetic applications. Comparable results have been reported in mangosteen peel and red pepper powder, where doses of 5 - 6 kGy effectively reduced microbial loads to acceptable safety thresholds [24,25].

Table 1 Microbial quality of Thanaka powder at various doses of gamma ray and X-ray irradiation.

Radiation source	Dose (kGy)	TVC (CFU/g)	TYC (CFU/g)	*B cereus*.
Thai FDA cosmetics regulations		1.0×10^3	1.0×10^2	ND
Non-irradiated	0	3.0×10^3	2.0×10^2	ND
Gamma ray	5	ND	ND	ND
	10	ND	ND	ND
	15	ND	ND	ND
	20	ND	ND	ND
X-ray	5	ND	ND	ND
	10	ND	ND	ND
	15	ND	ND	ND
	20	ND	ND	ND

Note: ND = Not detected (Detection limit >10 CFU/g). * TVC= Total viable bacterial count. TYC = Total yeasts and molds count.

Color evaluation

The influence of gamma ray and X-ray irradiation on the color parameters (L*, a* and b*) of Thanaka powder is summarized in **Table 2**. A significant dose-dependent reduction in lightness (L*) was observed following irradiation, as determined by Hunter's color measurements. The non-irradiated control exhibited an L* value of 79.13 ± 0.05, which decreased to 75.13 ± 0.00 and 75.35 ± 0.08 at 20 kGy for gamma ray and X-ray treatments, respectively. These results indicate progressive darkening with increasing radiation dose. The a* values, representing the red-green axis, increased significantly with irradiation, becoming less negative and indicating a shift from green toward red. In gamma ray-treated samples, the a* value increased from −1.08 ± 0.02 in the control to −0.45 ± 0.02 at 20 kGy, while X-ray treatment resulted in an increase to −0.35 ± 0.05 at the same dose. This trend reflects a reduction in green hue and a movement toward redness. Such color changes are commonly attributed to the degradation of photoactive compounds, including carotenoids and phenolic substances, which contribute to the characteristic hues of herbal powders. These bioactive pigments are often photoactive and sensitive to ionizing radiation, which can lead to oxidative breakdown or structural alteration. For example, studies on red pepper have demonstrated that irradiation reduces carotenoid and total phenolic contents, correlating with a loss of redness [26]. Because red carotenoids degrade more from radiation than yellow ones, the stable or slightly increased b* (yellowness) in some irradiated samples likely results from preserved yellow pigments or yellowish degradation products.

The observed decrease in L* and increase in a* values may result from pigment degradation or structural modifications of phenolic compounds caused by ionizing radiation. Additionally, Maillard-type reactions and oxidative processes may contribute to the slight darkening and red-shifting of the powder. Despite these alterations, the overall appearance of irradiated Thanaka powder remained within acceptable cosmetic quality standards. The findings support the hypothesis that reactive free radicals generated by irradiation interact with pigments and phytochemicals, leading to oxidation and degradation that manifest as visible color changes, such as darkening [27]. In contrast, b* values (yellowness) remained statistically unchanged across all radiation doses for both irradiation types, ranging from 17.17 to 17.91. As Thanaka powder naturally exhibits a yellow hue, the stability of the b* value is essential for

maintaining its visual identity. These findings suggest that while ionizing radiation may affect specific color attributes depending on the plant matrix and pigment composition, Thanaka powder retains acceptable color quality following gamma ray and X-ray irradiation.

Table 2 Effect of gamma ray and X-ray irradiation treatments on color values of Thanaka powder.

Dose (kGy)	Gamma ray			X-ray		
	L^*	a^*	b^*	L^*	a^*	b^*
Non-irradiated	79.13 ± 0.05^a	−1.08 ± 0.02^e	17.80 ± 0.02^a	79.13 ± 0.05^a	−1.08 ± 0.02^d	17.80 ± 0.02^a
5	78.68 ± 0.03^b	−0.75 ± 0.01^d	17.91 ± 0.03^a	75.02 ± 0.07^b	−0.67 ± 0.02^c	17.46 ± 0.58^a
10	77.31 ± 0.11^c	−0.69 ± 0.00^c	17.17 ± 0.06^a	75.62 ± 0.04^d	−0.64 ± 0.02^c	17.51 ± 0.04^a
15	76.81 ± 0.02^d	−0.60 ± 0.04^b	17.61 ± 0.02^a	75.80 ± 0.03^e	−0.44 ± 0.01^b	17.21 ±0.01^a
20	75.13 ± 0.00^e	−0.45 ± 0.02^a	17.69 ± 0.03^a	75.35 ± 0.08^c	−0.35 ± 0.05^a	17.31 ± 0.09^a

Note: The values expressed as mean ± SD of triplicate measurements.
Statistical differences were assessed using 1-way ANOVA followed by Duncan's test ($p < 0.05$).
Different letter in the same column indicates significant differences.

Arbutin and marmesin content

In this study, arbutin and marmesin concentrations in Thanaka powder were analyzed following gamma and X-ray irradiation at doses of 5, 10, 15, and 20 kGy (**Table 3**). The non-irradiated control contained 2.06 ± 0.00 mg/mL of arbutin. After irradiation, arbutin content ranged from 2.13 ± 0.00 to 2.16 ± 0.00 mg/mL for gamma ray and 2.07 ± 0.05 to 2.10 ± 0.00 mg/mL for X-ray. Marmesin was present at 0.14 ± 0.01 mg/mL in the non-irradiated. Post-irradiation levels ranged from 0.12 ± 0.00 to 0.14 ± 0.02 mg/mL for gamma rays and 0.12 ± 0.02 to 0.13 ± 0.02 mg/mL for X-ray, with no significant differences observed. The results of this study demonstrate that both gamma and X-ray irradiation at doses up to 20 kGy did not significantly alter the concentrations of arbutin and marmesin in Thanaka powder. However, a slight observed increase in arbutin levels, particularly at higher irradiation doses, may be attributed to Ionizing radiation induces covalent bonds in polysaccharides scission and generates highly reactive radicals, leading to cell wall loosening, increased porosity, and middle lamella disruption. These structural changes enhance solvent penetration and facilitate the release of intracellular constituents (e.g., phenolics, glycosides), thereby increasing the measurable yield of bioactive compounds in the extracts. This effect was observed in callus cultures of *Bergenia ciliata*, where arbutin concentration increased to 366.27 µg/g at 3.0 Gy compared to 228.52 µg/g in the control [28]. A similar pattern was also reported in gamma-irradiated Thanaka powder [9], suggesting that radiation-induced permeability may play a role in mobilizing arbutin from plant tissues. Moreover, gamma ray demonstrated slightly greater efficacy in enhancing arbutin content relative to X-ray irradiation. This is probably attributable to higher penetration capability and a broader energy spectrum, which facilitates the breaking down of covalent bonds and the generation of free radicals [29].

Conversely, the slight decrease in marmesin content at higher irradiation doses, may result from its structural sensitivity to radiolytic degradation. Marmesin, a coumarin derivative with a relatively stable conjugated ring system, may still undergo minor structural modifications upon exposure to high-energy radiation, such as ring opening or hydroxylation [30]. The results of this investigation support the notion that gamma and X-ray irradiation can improve the safety of Thanaka powder for consumer usage while maintaining its cosmeceutical performance.

Table 3 Effect of gamma ray and X-ray irradiation treatments on arbutin content and marmesin content of Thanaka powder.

Dose (kGy)	Gamma ray		X-ray	
	Arbutin (mg/mL)	Marmesin (mg/mL)	Arbutin (mg/mL)	Marmesin (mg/mL)
Non-irradiated	2.06 ± 0.00[a]	0.14 ± 0.01[a]	2.06 ± 0.00[a]	0.14 ± 0.01[a]
5	2.13 ± 0.00[a]	0.14 ± 0.02[a]	2.07 ± 0.05[a]	0.12 ± 0.02[a]
10	2.13 ± 0.00[a]	0.12 ± 0.02[a]	2.07 ± 0.05[a]	0.13 ± 0.02[a]
15	2.13 ± 0.05[a]	0.13 ± 0.00[a]	2.07 ± 0.05[a]	0.12 ± 0.02[a]
20	2.16 ± 0.00[a]	0.12 ± 0.00[a]	2.10 ± 0.00[a]	0.12 ± 0.02[a]

Note: The values expressed as mean ± SD of triplicate measurements. Statistical differences were assessed using 1-way ANOVA followed by Duncan's test ($p < 0.05$). Different letter in the same column indicates significant differences.

Tyrosinase inhibition and sun protection factor (SPF)

Thanaka powder is traditionally used in skincare due to its reputed anti-tyrosinase and UV-protective properties. These benefits are largely attributed to its phenolic compounds, which can inhibit tyrosinase activity and absorb UV radiation [31]. In the present study, the tyrosinase inhibitory activity of Thanaka powder was assessed following gamma ray and X-ray irradiation at doses of 5, 10, 15, and 20 kGy (**Table 4**). The non-irradiated control exhibited a tyrosinase inhibition of 8.39 ± 0.35%. For gamma ray-treated samples, values ranged from 8.10 ± 0.89% at 10 kGy to 8.85 ± 0.65% at 15 kGy. Similarly, X-ray-irradiated samples showed inhibition ranging from 8.10 ± 1.18% to 8.66 ± 1.18% across doses. Statistical analysis revealed no significant differences between irradiated and non-irradiated samples, suggesting that Thanaka's tyrosinase inhibitory activity remains stable after irradiation. This finding aligns with Aung *et al*. [32], who reported moderate tyrosinase inhibition by Thanaka extract in B16F10 murine melanoma cells, attributed to its rich phenolic content. Likewise, Han *et al.* [33] observed that 5 - 20 kGy of electron beam irradiation had no significant impact on the tyrosinase inhibitory activity of peanut shell extracts. Interestingly, other studies have demonstrated that low-dose gamma irradiation (e.g., 2 kGy) may even enhance tyrosinase inhibition by delaying enzymatic browning in food matrices such as king oyster mushrooms [34].

The sun protection factor (SPF) is a standard metric for evaluating the ability of topical products to absorb or block UVB radiation, which causes sunburn, erythema, and contributes to skin cancer [35,36]. In this study, the SPF of non-irradiated Thanaka powder was 6.74 ± 0.04, placing it in the "extra protection": Category (SPF 6 - 8) based on sunscreen protection classifications [37]. After gamma ray treatment, SPF values ranged from 6.55 ± 0.75 to 6.89 ± 0.02, and for X-ray-treated samples, from 6.17 ± 0.23 to 6.66 ± 0.40. These slight fluctuations were not statistically significant, confirming that SPF properties were not adversely affected by irradiation. These results support previous findings that Thanaka extract can absorb UVA rays, with maximum absorption at 335 nm [38], and now extend this to its UVB-protective potential. Several studies have shown that phenolic content correlates positively with SPF, reinforcing the idea that phenolic-rich plant extracts like Thanaka provide effective photoprotection [39]. Since gamma and X-ray irradiation did not significantly reduce arbutin or marmesin content (**Table 3**), the preservation of SPF values may be attributed to the stability of these bioactive compounds under irradiation conditions. The results, gamma ray and X-ray irradiation at doses up to 20 kGy do not significantly affect the tyrosinase inhibitory activity or SPF values of Thanaka powder. This highlights the stability of its cosmeceutical properties and supports the use of irradiation as a safe postharvest treatment method for microbial decontamination without compromising functional quality.

Table 4 Effect of gamma ray and X-ray irradiation treatments on tyrosinase inhibition (%) and sun protection factor (SPF) of Thanaka powder.

Dose (kGy)	Gamma ray		X-ray	
	Tyrosinase inhibition (%)	SPF	Tyrosinase inhibition (%)	SPF
Non-irradiated	8.39 ± 0.35[a]	6.74 ± 0.04[a]	8.39 ± 0.35[a]	6.74 ± 0.04[a]
5	8.18 ± 1.28[a]	6.89 ± 0.02[a]	8.66 ± 1.18[a]	6.66 ± 0.23[a]
10	8.10 ± 0.89[a]	6.55 ± 0.75[a]	8.10 ± 1.18[a]	6.17 ± 0.23[a]
15	8.85 ± 0.65[a]	6.69 ± 0.19[a]	8.28 ± 0.32[a]	6.33 ± 0.28[a]
20	8.29 ± 0.65[a]	6.76 ± 0.08[a]	8.47 ± 0.98[a]	6.62 ± 0.40[a]

Note: The values expressed as mean±SD of triplicate measurements. Statistical differences were assessed using 1-way ANOVA followed by Duncan's test ($p < 0.05$). Different letter in the same column indicates significant differences.

Total phenolic content (TPC)

Phenolic compounds are key plant-derived antioxidants that contribute to the cosmeceutical and nutraceutical value of herbal products. In this study, the total phenolic content (TPC) of Thanaka powder extract was evaluated following gamma and X-ray irradiation to assess the stability of these bioactives under ionizing radiation. As shown in **Figure 1**, the non-irradiated extract exhibited a TPC of 5.22 ± 0.06 mg GAE/g. Gamma-irradiated samples showed slightly higher values (5.31 ± 0.08 to 5.45 ± 0.18 mg GAE/g), while X-ray-treated samples ranged from 5.13 ± 0.06 to 5.29 ± 0.15 mg GAE/g. Although the differences were not statistically significant, these results indicate that phenolic compounds in Thanaka powder are stable under both gamma and X-ray irradiation up to 20 kGy. This finding aligns with previous work on *Peperomia pellucida* (L.), which showed no significant change in TPC following gamma irradiation at 10 kGy [40]. Several studies have reported enhanced phytochemical extraction or increased phenolic content following irradiation in various plant materials. For example, Aleksieva *et al.* [41] observed that gamma irradiation at 25 kGy increased polyphenol levels in walnuts by 44%, while crude olive pomace extracts irradiated at 6 kGy showed a 38.8% increase in total phenolic content compared to non-irradiated controls. These enhancements were attributed to radiation-induced disruption of plant cell walls, which facilitated the release of phenolic compounds [42]. However, this study did not observe a similar increase. This discrepancy may arise from differences in plant matrix composition, irradiation dose, or extraction methodology. Unlike those samples, the bioactive compounds in Thanaka powder, such as arbutin, marmesin, and phenolics, remained stable but were not enhanced by the applied radiation doses. This stability may be due to insufficient irradiation energy to break down cell structures and liberate additional phytochemicals. These results suggest that ionizing radiation may not only preserve but potentially enhance phenolic extractability in certain matrices, depending on plant structure and compound localization. Therefore, the stability of TPC in irradiated Thanaka further supports its suitability for decontamination processes without compromising antioxidant properties.

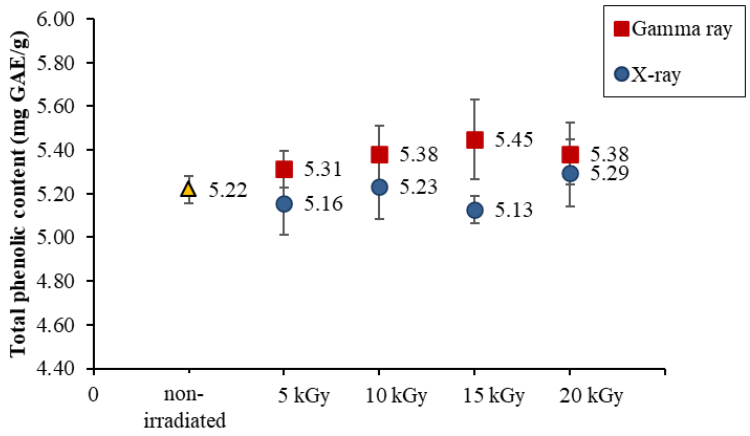

Figure 1 Total phenolic content expressed as mg of gallic acid equivalents (GAE)/g crude extract of Thanaka powder at various doses of gamma and X-ray irradiation. Error bars represent standard deviations (n = 3).

Antioxidant activities

The antioxidant activity of Thanaka powder was assessed using DPPH and FRAP assays. The DPPH assay measures the free radical scavenging capacity of antioxidants, expressed as mg ascorbic acid equivalent (AAE) per gram of extract. As shown in **Figure 2(a)**, gamma-irradiated samples exhibited scavenging activity ranging from 1.78 ± 0.09 to 1.90 ± 0.02 mg AAE/g, while X-ray-treated samples ranged from 1.78 ± 0.09 to 1.92 ± 0.08 mg AAE/g. No statistically significant differences were observed compared to the non-irradiated control, indicating that antioxidant activity was maintained following irradiation. The stability of DPPH scavenging activity suggests that irradiation doses up to 20 kGy do not degrade the antioxidant constituents in Thanaka powder. This effect is likely due to the presence of phenolic and coumarin compounds, such as marmesin, which possess hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals [43]. Previous studies have reported similar findings in other plant matrices, where irradiation did not negatively impact DPPH activity. For example, *Peperomia pellucida* extracts retained antioxidant activity after gamma irradiation at 10 kGy [40].

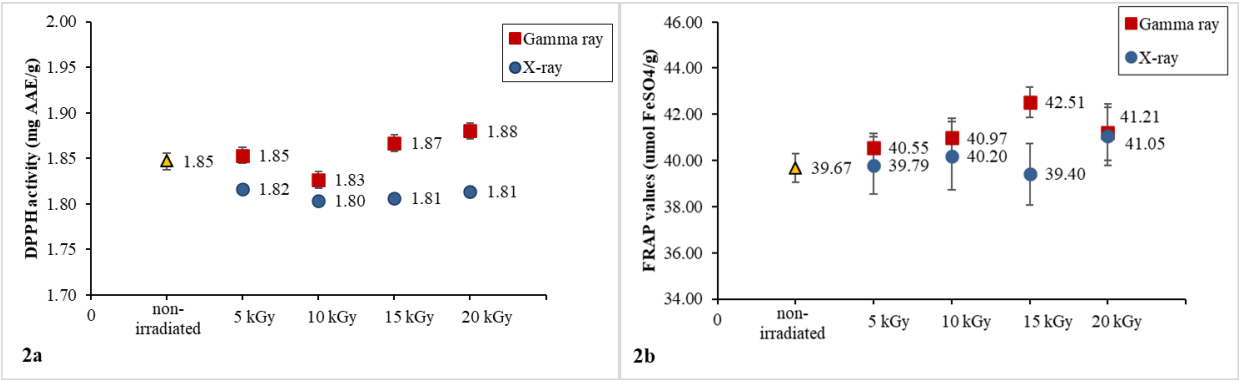

Figure 2 Antioxidant activities of Thanaka powder at various doses of gamma and X-ray irradiation. (a) DPPH radical scavenging activity expressed as mg ascorbic acid equivalent (AAE)/g crude extract and (b) ferric reducing antioxidant power (FRAP) expressed as µmol $FeSO_4$ equivalent/g crude extract of Thanaka powder**.** Error bars represent standard deviations (n = 3).

The FRAP assay evaluates the antioxidant capacity of a sample by measuring its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. As shown in **Figure 2(b)**, the FRAP value of non-irradiated Thanaka powder was 39.67 ± 0.63 µmol $FeSO_4$/g. Gamma-irradiated samples showed slightly increased values, ranging from

40.55 ± 0.64 to 42.51 ± 0.66 μmol $FeSO_4$/g, while X-ray-treated samples ranged from 39.40 ± 1.32 to 41.05 ± 1.26 μmol $FeSO_4$/g. However, none of the differences were statistically significant ($p > 0.05$), indicating that the ferric reducing capacity remained stable after irradiation. These findings are consistent with previous reports, such as in mangosteen peel extract, where both gamma and electron beam irradiation had no significant effect on FRAP or DPPH values [24]. The slight upward trend in FRAP values following gamma irradiation may be due to improved extractability of phenolic antioxidants, as ionizing radiation can disrupt cell wall structures and facilitate the release of bound phytochemicals [28].

Conclusions

Gamma ray and X-ray irradiation at doses ranging from 5 to 20 kGy effectively reduced the microbial load of Thanaka powder to levels compliant with Thai FDA cosmetic safety standards, ensuring hygienic quality. Both irradiation methods demonstrated comparable efficacy in microbial decontamination. The non-irradiated sample exceeded Thai FDA cosmetic limits for TVC (3.0×10^3 CFU/g) and TYC (2.0×10^2 CFU/g), with *Bacillus cereus* undetected. Gamma and X-ray irradiation at doses ≥ 5 kGy effectively reduced microbial counts to below detectable levels. Importantly, irradiation up to 20 kGy did not significantly affect key bioactive compounds, including arbutin and marmesin, nor did it alter total phenolic content or antioxidant capacity as measured by DPPH and FRAP assays. Tyrosinase inhibitory activity and sun protection factor (SPF) were also retained, confirming the preservation of Thanaka powder's functional cosmeceutical properties. Thanaka powder's tyrosinase inhibition (~8.4%) and SPF (6.2 - 6.9) remained stable after gamma and X-ray irradiation (5 - 20 kGy), likely due to preserved arbutin and marmesin levels. Although minor color changes such as dose-dependent darkening and a shift from green toward red hues were observed, these did not affect the overall cosmetic quality or characteristic appearance of the powder. This study confirms that gamma ray and X-ray irradiation are safe and effective postharvest treatments for microbial control in Thanaka powder, maintaining its bioactive and antioxidant integrity. Furthermore, X-ray irradiation presents a practical alternative to conventional gamma sources, offering flexibility in processing environments. The stability of Thanaka's functional properties after irradiation supports the safe application of these technologies in large-scale herbal cosmetic production, ensuring compliance with regulatory standards for product safety and quality.

Acknowledgements

The authors are grateful to Thailand Science Research and Innovation for the financial support and Irradiation center, Thailand Institute of Nuclear Technology (Public Organization) for X-ray irradiation process.

Declaration of Generative AI in Scientific Writing

During the preparation of this manuscript, the authors utilized QuillBot to improve the language's clarity and grammar. Following the application of this tool/service, the authors thoroughly reviewed and edited the content as necessary and take full responsibility for the final published version.

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Sirilak Chookaew: Conceptualization, Methodology, Investigation, Data curation, and Writing - Original Draft. **Wachiraporn Pewlong**: Project administration and Investigation. **Jaruratana Eamsiri**: Investigation. **Surasak Sajjabut**: Investigation. **Khemruji Khemtong**: Investigation. **Ratchaneeporn Photinam**: Visualization, and Writing - Reviewing and Editing.

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