

RESEARCH ARTICLE

Open Access



# Total phenolic content and in vitro photoprotective activity of ethanolic extract of torch ginger (*Etilingera elatior*)

Fawnia Alma Arabella Fahmi<sup>1</sup>, Rehana<sup>1\*</sup>, Beti Pudyastuti<sup>1</sup>

## ABSTRACT

**Background:** Previous research highlighted the photoprotective potential of various extracts from torch ginger leaves, but the efficacy of ethanolic extracts, particularly from leaves and other plant parts, remained unexplored.

**Objective:** This study aimed to assess the total phenolic content and SPF value of ethanolic extracts from torch ginger leaves, flowers, and stems, comparing these with a known photoprotective agent, oxybenzone.

**Method:** Ethanolic extraction was performed on approximately 200 grams of each plant part. The total phenolic content was determined using the Folin-Ciocalteu method, while SPF values were measured spectrophotometrically between 290 and 320 nm. Statistical analysis involved Kruskal-Wallis tests followed by post-hoc Fisher's LSD.

**Results:** The leaf extract exhibited the highest phenolic content ( $483.788 \pm 2.57$  mg GAE/g) and SPF value at 1000 ppm, surpassing that of flowers, stems, and the positive control, oxybenzone. The correlation between phenolic content and SPF value across plant parts was statistically significant.

**Conclusion:** The ethanolic extract of torch ginger leaves shows superior photoprotective potential, indicated by its high phenolic content and SPF value, suggesting its promising application in natural sunscreen formulations up to the permissible active substance limit of 6% set by BPOM.

**Keywords:** torch ginger, sun protection factor, total phenol, photoaging, ethanolic extract

## Introduction

Torch ginger, or *kecombrang* (*Etilingera elatior* (Jack) R.M.Sm.), has traditionally been used for its anti-aging properties [1]. This activity is attributed to phenolic compounds as well as alkaloids and tannins. Phenols are known for their chromophores and auxochromes, which can absorb UVA and UVB rays. Furthermore, the free electron pairs in auxochromes may mitigate free radicals resulting from UV exposure [2].

Previous studies have assessed the sun protection factor (SPF) of water, ethyl acetate, and *n*-hexane extracts from torch ginger leaves. Notably, the *n*-hexane extract demonstrated a significant SPF value of 17.579

$\pm 2,495$  at a concentration of 300 ppm [3]. However, the photoprotective potential of leaf ethanol extracts remains unexplored. Given ethanol's appropriate polarity for extracting phenolic compounds and its lower toxicity compared to *n*-hexane, it is hypothesized that ethanol extracts may exhibit higher SPF activity due to a more effective solvation of photoprotective phenolic compounds.

Furthermore, phenolic compounds' presence in other plant parts, such as stems and flowers, suggests that their ethanol extracts may also possess SPF activity. Therefore, assessing the total phenolic content and SPF activity across these plant parts is essential to identify the most promising photoprotective agent.

This study aims to measure the total phenolic content and SPF activity of ethanol extracts from the leaves, stems, and flowers of torch ginger. By comparing these findings to oxybenzone, a commonly used

Department of Pharmacy, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto 53123, Indonesia

\*Corresponding author: Jl Dr. Soeparno Karangwangkal Purwokerto 53123, Indonesia. E-mail: [rere.rehana@gmail.com](mailto:rere.rehana@gmail.com)

sunscreen ingredient, we seek to establish a correlation between phenolic content and photoprotective efficacy. Using oxybenzone as a positive control allows for a comparative analysis of the extracts' SPF values in relation to a standard sunscreen component.

## Methods

### Preparation of ethanolic extract

Torch ginger plants were collected from Tamansari Village, Karanglewas District, Banyumas Regency, Indonesia. Specimen identification was conducted at the Environmental Laboratory, Faculty of Biology, Jenderal Soedirman University, Indonesia, confirming the species as *Etlingera elatior*. Approximately 200 grams of each plant part (leaves, stems, and flowers) in powdered form underwent maceration in 1 liter of 70% ethanol p.a (Merck) three times. The resulting filtrate was evaporated over seven days until clear, followed by further reduction to a thick extract using a rotary evaporator (Buchi B. 480) at 40°C.

### Measurement of total phenolics

**Determination of operating time:** A solution containing 0.1 mL of 502 ppm gallic acid, three drops of Folin-Ciocalteu reagent, 2 mL of 7.5% sodium carbonate, and distilled water up to 10 mL was incubated. Absorbance was measured at 765 nm for 100 minutes using a UV-Visible spectrophotometer (Shimadzu UV-1780), based on protocols established in previous studies [4].

**Determination of maximum wavelength:** A similar solution was prepared and incubated for 80 minutes (operating time determined by previous step result) in the dark. Absorbance was then measured across a wavelength range of 400 nm to 800 nm to identify the maximum absorption wavelength.

**Determination of the gallic acid standard curve:** Solutions of varying concentrations of gallic acid (200.8; 401.6; 502; 602.4; and 803.2 ppm) were prepared by adding three drops of Folin-Ciocalteu reagent, 2 mL of 7.5% sodium carbonate, and distilled water up to 10 mL. After 80 minutes of dark incubation, absorbance at 767 nm was measured to construct a standard curve of absorbance as a function of gallic acid concentration.

**Determination of total phenolic content in extracts:** For each torch ginger part extract (leaf, flower, stem) at 1000 ppm, 0.1 mL was mixed with

three drops of Folin-Ciocalteu reagent, 2 mL of 7.5% sodium carbonate, and distilled water up to 10 mL. The mixture was incubated in the dark for 80 minutes before measuring the absorbance at 767 nm. This measurement was performed in triplicate for each extract.

The total phenolic content was calculated using the formula: Total phenolics =  $(x \times V \times df) / \text{mass of sample}$ , where  $x$  = concentration in ppm,  $V$  = sample volume in liters,  $df$  = dilution factor, and mass of sample in grams.

### SPF value measurement

The absorbance of each solution—comprising leaf, flower, stem, and oxybenzone extracts dissolved in 70% ethanol, at concentrations of 100, 200, 300, 500, and 1000 ppm—was recorded across the UVB range (290 to 320 nm) at 5 nm intervals. The SPF value for each extract was calculated using the following equation:

$$\text{SPF}_{\text{spectrophotometric}} = CF \times (EE(\lambda) \times I(\lambda) \times Abs(\lambda))$$

where  $EE(\lambda)$  is the erythema effect spectrum,  $I(\lambda)$  is the light intensity spectrum,  $Abs(\lambda)$  is the absorbance of the sample at wavelength  $\lambda$ , and  $CF$  is the correction factor, set at 10. The product of  $EE$  and  $I$  for each wavelength is provided in Table 1, according to reference [5].

**Table 1.** Erythema effect spectrum and light intensity [5]

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

### Data analysis

The statistical analysis was conducted using SPSS software version 27.0.1.0. Both SPF and total phenolic content data underwent tests for Levene's homogeneity of variances and the Kolmogorov-Smirnov test for normality. The results indicated that the data were neither homogeneous nor normally distributed.

Consequently, non-parametric analysis was employed, utilizing the Kruskal-Wallis test to discern differences between groups. Where significant differences were found, post-hoc comparisons were made using Fisher's Least Significant Difference (LSD) test.

## Results

### Yields of ethanolic extracts

The extraction process resulted in yields of 16.003% for leaves, 10.013% for flowers, and 8.699% for stems. These yields indicate the efficiency of ethanol in extracting compounds from different plant parts, with leaves showing the highest extraction yield.

### Determination of optimal incubation time

Absorption measurements conducted at 765 nm across various incubation times (0, 15, 30, 45, 60, 70, 75, 80, 85, 90, 95, and 100 minutes) identified an optimal operating time of 80 minutes for maximal absorption, as illustrated in Figure 1.

### Maximum wavelength for absorption

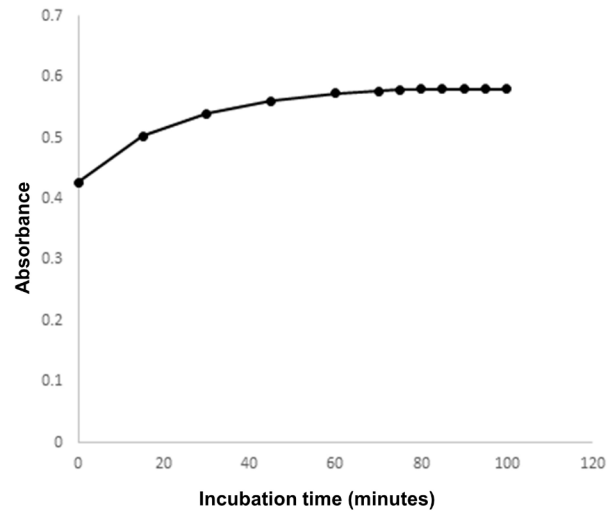
Further analysis within the 400 to 800 nm wavelength range, following an 80-minute incubation, pinpointed 767 nm as the wavelength of maximum absorption (Figure 2). This result is critical for accurately measuring phenolic content, as it confirms the optimal wavelength for subsequent analyses.

### Gallic acid standard curve

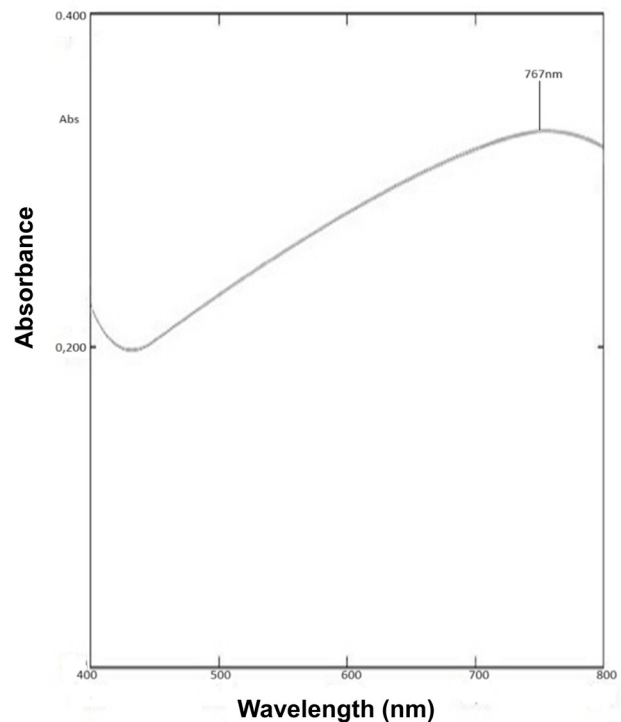
The relationship between gallic acid concentration and uptake was quantified, yielding a linear equation  $Y=0.0012x-0.066$  with an  $R^2=0.9895$  (Figure 3). This high  $R^2$  value indicates a strong correlation between concentration and absorption, validating the method for estimating phenolic content.

### Total phenolic content

Measurements of total phenolic content in the leaf, flower, and stem extracts yielded  $483.788 \pm 2.57$  mg GAE/g,  $261.160 \pm 1.34$  mg GAE/g, and  $219.638 \pm 0.41$  mg GAE/g, respectively. These results demonstrate significant variations in phenolic content across different plant parts, with leaves exhibiting the highest content.



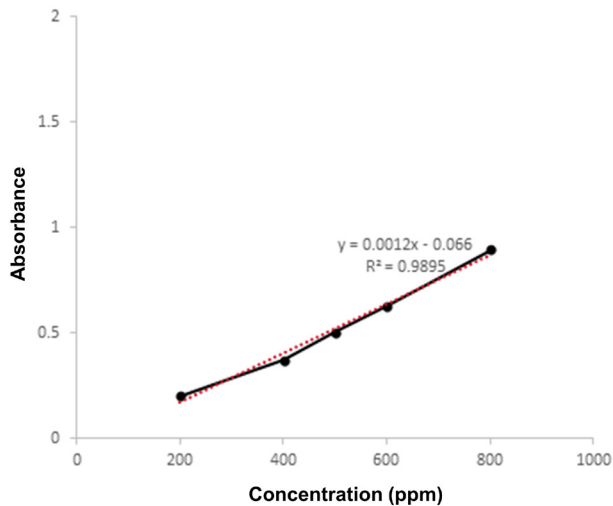
**Figure 1.** Relationship curve of gallic acid absorbance as a function of incubation time



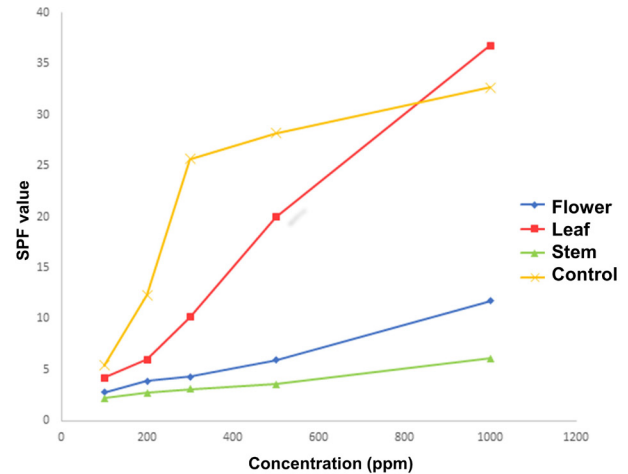
**Figure 2.** Maximum wavelength measurement

### SPF measurement results

The SPF values of the extracts and controls, as a function of concentration, are detailed in Figure 4. At a concentration of 1000 ppm, the SPF values for the flowers, leaves, stems, and control were  $11.760 \pm 0.05$ ,  $36.803 \pm 1.52$ ,  $6.105 \pm 0.08$ , and  $32.680 \pm 0.24$ , respectively.



**Figure 3.** Gallic acid absorbance relationship curve as a function of concentration



**Figure 4.** Relationship curve of SPF value as a function of concentration

## Discussion

The observation that the yield from leaf ethanolic extracts is higher than that from flowers and stems can be attributed to the leaves' role as the primary site of photosynthesis in plants. Leaves are rich in both primary and secondary metabolites, produced as part of the plant's natural physiological processes. Secondary metabolites, including phenolic compounds, are abundant in leaves due to their involvement in plant defense mechanisms against pathogens, UV radiation, and herbivores [6].

Gallic acid is a commonly used benchmark in phenolic assays due to its well-characterized properties and prevalence within the phenolic compound group. The determination of the optimal incubation time was crucial for ensuring the complete development of the colorimetric reaction between phenolic compounds and the Folin-Ciocalteu reagent. The incubation time was established by monitoring the absorption changes post-incubation in the dark, at a wavelength previously identified [4]. This step ensured that the color complex formation, indicative of phenol presence, was fully developed, providing a reliable basis for subsequent analyses.

Following the establishment of the incubation time, the maximum wavelength for absorption (767 nm) was determined through spectrophotometric analysis within the visible range (400-800 nm). This wavelength, slightly deviating from prior research [4], falls within the acceptable variation limit set by the Indonesian Pharmacopoeia of  $\pm 3$  nm [7].

The observed differences in total phenolic content among the plant parts were statistically significant ( $p = 0.027$ ), with the leaf extracts exhibiting the highest phenolic levels, followed by flowers and stems. This disparity is attributed primarily to the varying degrees of sunlight exposure among these parts. Phenolic compounds serve as a protective adaptation in plants, mitigating the detrimental effects of UV radiation by absorbing the harmful rays and acting as antioxidants and scavengers of reactive oxygen species (ROS) [2]. Furthermore, environmental conditions such as temperature, moisture, soil salinity, and mineral content also contribute to the diversity in phenolic content across different plant parts [8].

Comparative analysis with previous studies highlights ethanol's superiority as an extraction solvent for phenolic compounds. For instance, the total phenolic content in leaf ethanolic extracts was reported at  $483.788 \pm 2.57$  mg GAE/g, significantly higher than the  $246.52 \pm 0.26$  mg GAE/g observed in water extracts [9]. Similarly, the phenolic content in flower ethanolic extracts surpassed that in ethyl acetate extracts, emphasizing ethanol's efficacy in solubilizing phenolic compounds due to its structural compatibility and solvent properties. This observation suggests that the polar nature of flavonoids in torch ginger is better matched with ethanol's polarity.

The SPF measurement further supports the phenolic content findings, with leaf extracts showing the highest SPF values, significantly differing from those of flowers ( $p < 0.001$ ) and stems ( $p = 0.013$ ), yet comparable

to the synthetic sunscreen oxybenzone ( $p = 1.000$ ). Notably, the SPF value of torch ginger leaf ethanolic extract demonstrated potential for further increase within the maximum concentration limits allowed by the Indonesian Food and Drug Authority (Badan POM), set at 6% or 60,000 ppm [7]. This trend contrasts with oxybenzone, where SPF value increment diminishes beyond 300 ppm. Given oxybenzone's role as an antioxidant and ROS scavenger [2], its comparison as a positive control underscores the promising photoprotective efficacy of torch ginger extracts.

The alignment in yield value, total phenolic content, and SPF value across plant parts (leaves, flowers, stems) corroborates the accuracy of the measurement methodologies employed in this study. This consistency reinforces the reliability of the findings and highlights the potential of torch ginger, particularly its leaves, in photoprotective applications.

## Conclusion

The study demonstrates that the ethanolic extract of torch ginger leaves contains the highest level of phenolic compounds compared to the flower and stem extracts. This finding is consistently reflected in the SPF measurements, where the leaf extract not only surpasses the other plant parts at a concentration of 1000 ppm but also exhibits a higher SPF value than the positive control. This correlation between total phenolic content and SPF value underscores the significant photoprotective potential of the leaf ethanolic extract. Importantly, the study suggests that the SPF value of the leaf extract could be further optimized up to the maximum concentration of active substances permitted by the Indonesian Food and Drug Monitoring Agency (BPOM), which is 6%. These results highlight the promising utility of torch ginger leaf ethanolic extract as a natural photoprotective agent, warranting further research and development towards its application in sunscreen formulations.

## Acknowledgment

Authors acknowledge Department of Pharmacy Universitas Jenderal Soedirman for providing facilities.

## Conflict of Interest

None.

## Author contributions

FAAF, R, BP conceptualized the study design; FAAF investigated the data; FAA and R wrote original draft, R reviewed and edited the final version, R supervised all experiment. All authors have read the final manuscript.

Received: 27 February 2024

Revised: 24 March 2024

Accepted: 24 March 2024

Published online: 27 March 2024

## References

1. Silalahi M (2017) *Etlingera elatior* (Jack) R. M. Smith: Manfaat dan Aktivitas Biologi. Skripsi. Universitas Negeri Yogyakarta.
2. Del Valle JC, Buide ML, Whittall JB, Valladares F, Narbona E (2020) UV radiation increases phenolic compound protection but decreases reproduction in *Silene littorea*. *PLoS ONE* 15, e0231611. <https://doi.org/10.1371/journal.pone.0231611>
3. Pramiastuti, O (2019) Penentuan nilai spf (sun protection factor) ekstrak dan fraksi daun kecombrang (*Etlingera elatior*) secara in vitro menggunakan metode spektrofotometri. *JIPP* 8(14). <https://doi.org/10.30591/pjif.v8i1.1281>
4. Martins GR, Monteiro AF, do Amaral FRL, da Silva AS (2021) A validated Folin-Ciocalteu method for total phenolics quantification of condensed tannin-rich açai (*Euterpe oleracea* Mart.) seeds extract. *J. Food Sci. Technol.* 58: 4693-4702. <https://doi.org/10.1007/s13197-020-04959-5>
5. Prakash PKT, Lokesh PNKS, Manral K (2015) A simple and rapid method developed to determine the Sun protection factor (SPF) by using UV-visible spectrophotometer for topical formulations. *Journal of Research & Method in Education*, 5(1): 1-5.
6. Senduk TW, Montolalu, LADY, Dotulong V (2022) The rendement of boiled water extract of mature leaves of mangrove (*Sonneratia alba*). *Jurnal Perikanan dan Kelautan Tropis*, 11(1): 9-15. <https://doi.org/10.35800/jpkt.11.1.2020.28659>
7. Badan Pengawas Obat dan Makanan (2022) Peraturan Badan Pengawas Obat dan Makanan Nomor 17.
8. Li Y, Kong D, Fu Y, Sussman MR, Wu H, (2020) The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol. Biochem.* 148: 80-89. <https://doi.org/10.1016/j.plaphy.2020.01.006>
9. Whangsomnuek N, Mungmai L, Mengamphan K, Amornlerdpison D (2019) Bioactive compounds of aqueous extracts of flower and leaf of *Etlingera elatior* (Jack) RM Sm. for cosmetic application. *Maejo International Journal of Science and Technology*, 13(3): 196-208.