EFFECT OF DRYING METHOD ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF BLACK GINGER (KAEMPFERIA PARVIFLORA WALL. EX BAKER) SLICES IN DAK LAK, VIETNAM

Hoang Nhat Phi [(1)], **Pham Thi My Tram** [(1)]
(1) Institute of Green and Sustainable Technology, Thu Dau Mot University
Corresponding author: tramptm@tdmu.edu.vn

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Abstract

The herbal plant known as black ginger (Kaempferia parviflora Wall. Ex Baker) is a member of the Zingiberaceae family and is extensively distributed in tropical and subtropical regions of Asia. Traditionally, black ginger is used as a health-enhancing herb to relieve joint pain, digestive disorders, and infections.

This study aimed to assess how drying method (sunlight, dried in an oven) affected the moisture content, total phenolic content, and antioxidant activity of slices of black ginger root and rhizome. Total phenolic content was measured using the UV-vis spectrophotometry method with a gallic acid standard. The DPPH free radical scavenging experiment was also used to assess the extracts' antioxidant potential.

Phytochemical screening results showed that black ginger rhizomes and roots had many secondary metabolites, such as alkaloids, flavonoids, polyphenols, and tannins. The highest total phenolic content was found in sliced black ginger rhizomes dried at 60°C (69.15 mg GAE/g extract). Moreover, black ginger samples all had relatively good antioxidant activities, with IC50 values 42.34 to 67.14 times higher than the IC50 of ascorbic acid. The investigation results on drying temperature's effects on TPCs, and the antioxidant activities of black ginger rhizomes and roots support knowledge and experience in using dried black ginger as raw materials for food and pharmaceutical industries.

Keywords: Antioxidant, black ginger, drying, Kaempferia parviflora Wall. Ex Baker, phenolic, Zingiberaceae

1. Introduction

The genus *Kaempferia* has about 66 species that are found in tropical and subtropical regions of Asia, including China, India, Vietnam, Thailand, Laos, and Cambodia. (Loan et al., 2023). Engelbert Kaempfer (1651–1716), a German explorer, naturalist, and writer, is honored by this generic name. Several species of *Kaempferia* are widely used worldwide as flavorants, spices, and traditional medicines for infectious diseases, wound infection, cough, pain, and digestion disorders (Khanh and Binh, 2021; Singh et al., 2023). The plants belonging to *Kaempferia* contain many essential oils, diterpenes, phenolic compounds, and flavonoids (Singh et al., 2023). Therefore, plants of the genus *Kaempferia* have become a valuable medicinal material for new natural treatment applications for humans.

Black ginger (*Kaempferia parviflora*) is a member of the genus *Kaempferia*, Zingiberaceae family, and is a herbaceous plant living in groups (bushes), originating from Thailand (Park et al., 2021). According to traditional medicine, black ginger is a herb that helps improve health, treat bone and joint diseases, increase sexual desire, and treat stomach pain and duodenal ulcers (Banjerdpongchai et al., 2008). In Vietnam, black ginger is found in provinces such as Thanh Hoa, Gia Lai, Dak Lak, An Giang, and Lai Chau (Nguyen et al., 2023; Tuan and Sanh, 2020; Huyen et al., 2024). Its rhizomes are dark purple, and unlike common ginger, it is a perennial herb that reaches a height of around 90 cm (Figure 1).

Figure 1. Black ginger (*Kaempferia parviflora* Wall. Ex Baker) in M'Drak district, Dak Lak province, A: Morphological characteristics; B: Rhizomes and roots; C: Rhizomes; D: Flowers

(Source: Author, 2025)

Several studies have shown that black ginger rhizomes contain many compounds, such as flavonoids, monoterpene, sesquiterpene, steroid, phenolic (Phung et al., 2021; Atom et al., 2022), with antioxidant and anti-microbial/fungal activities (Atom et al., 2022; Nguyen et al., 2023), anti-osteoporotic (Thao et al., 2016), anticancer activity (Banjerdpongchai et al., 2008; Thuy et al., 2023); anti-inflammatory (Tewtrakul et al., 2009), skin protective efficacy (Phung et al., 2021), anti-obesogenic, and anti-diabetic activities (Prabakaran et al., 2021). Ginger rhizomes and roots usually have a moisture content of 85-95%, so microorganisms easily damage them when stored fresh for a long time. Therefore, finding methods to preserve herbs, including black ginger, is extremely necessary. Drying methods are often used to preserve medicinal herbs, especially under sunlight. However, different drying temperatures will affect the compounds and the activity of medicinal powder (Quang et al., 2016; Muhammad et al., 2022). Currently, very few studies focus on finding the optimal temperature to obtain high-quality black ginger powder and protect its bioactive compounds. However, studies on compounds and biological activities from black ginger rhizomes grown in MDrak district, Dak Lak province, have not been reported.

This study was conducted to find appropriate dry conditions to obtain the highest concentration of phenolics and antioxidant activity of powder from black ginger plant in M'Drak district, Dak Lak province, Vietnam, providing further evidence of the antioxidant activity of black ginger, aiming at the application and preparation of products in the food or pharmaceutical fields to support community health.

2. Materials and methods

2.1. Materials

Black ginger (*Kaempferia parviflora* Wall. Ex Baker), including fresh rhizomes and roots in M'Drak district, Dak Lak province, Vietnam, were transported to Thu Dau Mot University laboratory, washed thoroughly with normal water to remove dust and finally rinsed with distilled water (Figure 2). They were then sliced into pieces (Figure 3), dried, ground into fine powder and extracted to survey qualitative analysis, total phenolic content, and antioxidant activity.

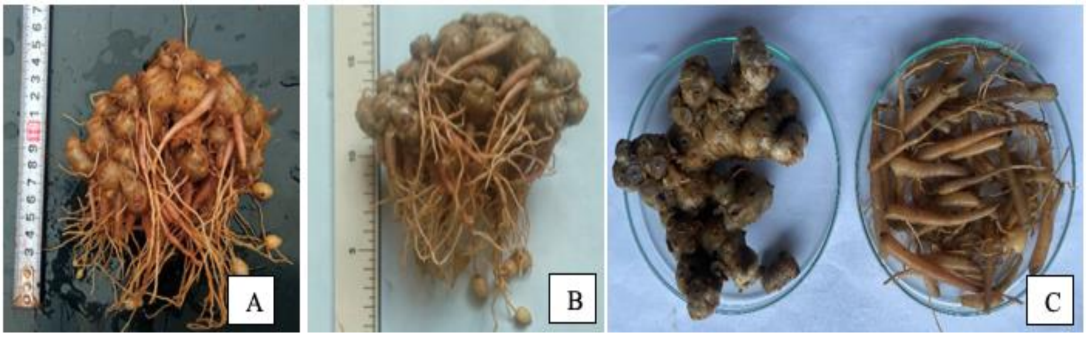

Figure 2. Fresh rhizomes and roots: A) Before washed, B) After washed, C) Separated

(Source: Author, 2025)

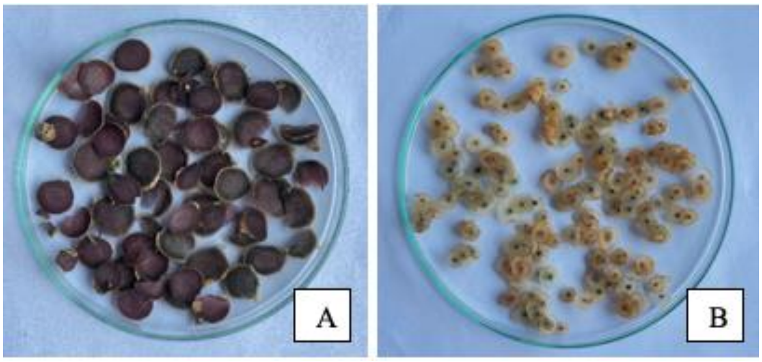

Figure 3. Fresh black ginger was sliced into pieces: A) Rhizomes, B) Roots

(Source: Author, 2025)

2.2. Chemicals

Ethanol (99.5% purity, CEMACO - Vietnam) was used for extraction. Lead (II) acetate trihydrate ($Pb(CH_3COO)_2.3H_2O$), ferric trichloride anhydrous ($FeCl_3$), natri hydroxide, and gelatin (Xilong, China) were used for qualitative analysis. Ascorbic acid

(VWR Chemicals) and gallic acid (Bio Basic, Canada) were used as standards in spectrophotometry analysis, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich was used to analyze antioxidant activity. The study was conducted at Thu Dau Mot University laboratory in Vietnam from November 2024 to April 2025.

2.3. Instrumentation

In this study, a Memmert drying oven was used for drying samples. Firstek B601D reciprocal shaker bath was used to extract, and Biochrom WPA Biowave II spectrophotometer was used to analyze total phenolic content and antioxidant activity.

2.4. Methods

2.4.1. Study on the effect of drying temperature on moisture content, total phenolic content, and antioxidant activity of black ginger rhizome and root slices

The fresh rhizomes and roots from black ginger were sliced into pieces around 1 ± 0.2 mm thickness using a scalpel (Figure 3), exposed to sunlight (starting from 10 a.m. with temperatures between 37 and 45°C), and dried in an oven at 60°C and 70°C until the moisture contents of the samples were below 5%. The samples were ground into powder form by using a blender. Then, these samples were boiled with ethanol 99.5% reciprocal shaker bath at 70°C. After three hours, the mixtures were filtered, concentrated, and stored at 4°C (Mustafa and Chin, 2023) until further use to determine their total phenolic contents (TPCs) and antioxidant activity.

2.4.2. Phytochemical screening for secondary metabolites in black ginger extracts

The powders of dried rhizomes and roots from black ginger (1.0 g) were added to 20 mL of ethanol 99.5% in a 100 mL Erlenmeyer flask, then boiled in a reciprocal shaker bath at 70°C for three hours. The mixtures were filtered and tested, as shown in Table 1.

Table 1. Phytochemical screening for secondary metabolites in black ginger extracts

Secondary metabolite	Qualitative chemical reaction	Signs of recognition
Alkaloids	0.5 mL extract + HCl 1.5% + 3-4 drops of Wagner reagent	Reddish-brown precipitate
Flavonoids	0.5 mL extract + 0.5 mL $Pb(CH_3COO)_2$	Yellow precipitate
Polyphenols	0.5 mL extract + 3-4 drops of $FeCl_3$ 10%	Bluish-green appearance
Saponins	0.5 mL extract + 5 mL H_2O + 3-4 drops of ethano l 99%,, shake and let stand for 15 minutes.	Froth appearance
Tannins	0.5 mL extract + 5 drops gelatin 1%	White precipitate

(Source: Apoorva et al., 2021; Nhung and Quoc, 2024)

2.4.3. Determination of moisture content

The slices of black ginger (1 g) were dried in an oven at 105°C for 5 hours. The moisture content (W_{H2O}) was expressed as a percentage mass fraction, according to equation (1): $W_{H20} = (m_0 - m_1)*100/m_0$ (1), where m_0 is the mass of the original fresh sample, in grams (four decimals), m_1 is the mass of the sample after drying, in grams (four decimals) (Chryssou et al., 2018).

2.4.4. Determination of total phenolic contents (TPCs)

The Folin-Ciocalteu method, which measures spectrophotometrically at 765 nm, was used to estimate TPCs (Quan et al., 2016). Mix 1 mL of the sample with 5 mL of 10% Folin-Ciocalteu and 4 mL of 7.5% Na_2CO_3 solution. Then, cap, shake, leave room temperature for an hour in the dark, and measure the absorbance at 765 nm. The dried extracts were prepared as solutions in the ethanol solvent at a 500 µg/mL concentration. A standard calibration curve was produced using gallic acid at 0, 10, 20, 30, 40, and 50 µg/mL values. The findings are given in milligrams of gallic acid equivalent (GAE) per gram of extract.

2.4.5. Determination of antioxidant activity using the DPPH scavenging test

The antioxidant activity of ethanol extracts of black ginger (rhizomes and roots) was determined based on the DPPH radical scavenging method. Briefly, 0.5 mL of 0.6 mM DPPH diluted in ethanol was added to 3 mL of 99.5% ethanol and 0.5 mL of extract (125 – 2000 µg/mL). The reaction mixture was incubated at room temperature for half an hour in the dark. The absorbance was measured at 517 nm. Ascorbic acid (3.125 – 50 µg/mL) was a reference standard. Absolute ethanol was a negative control (Hung et al., 2014).

DPPH radical scavenging activity as a percentage was determined using the following equation (3): $\%\text{Inhibition} = (1 - \frac{\text{Abs sample}}{\text{Abs control}}) \times 100\%$ (3), where Abs_{sample} was the sample absorbance, $Abs_{control}$ was the control absorbance (Hung et al., 2014).

2.4.6. Statistical analysis

Experimental data were analyzed using Stagraphics Centurion XV software. The results were presented as mean ± standard deviation (SD) with three replications. One-way analysis of variance was applied to analyze statistical significance ($p < 0.05$).

3. Results and discussion

3.1. Phytochemical analysis result of secondary metabolites in black ginger extracts

The presence of bioactive compounds from black ginger rhizome and root extracts was recorded in Figure 4 and Table 2.

When the reagents were added to the extracts (test tubes A and B), a reddish-brown precipitate (test tube No. 1), a yellow precipitate (test tube No. 2), a dark blue (test tube No. 3), and a white precipitate (test tube No.5) appeared. Test tube No.4 did not appear froth (Figure 4).

Table 2. Phytochemical result of secondary metabolites obtained in black ginger extracts

Test	Dried sample	
	Rhizomes	Roots
Alkaloids	+	+
Flavonoids	+	+
Polyphenols	+	+
Saponins	-	-
Tannins	+	+

(+) presence, (-) absence

(Source: Author, 2025)

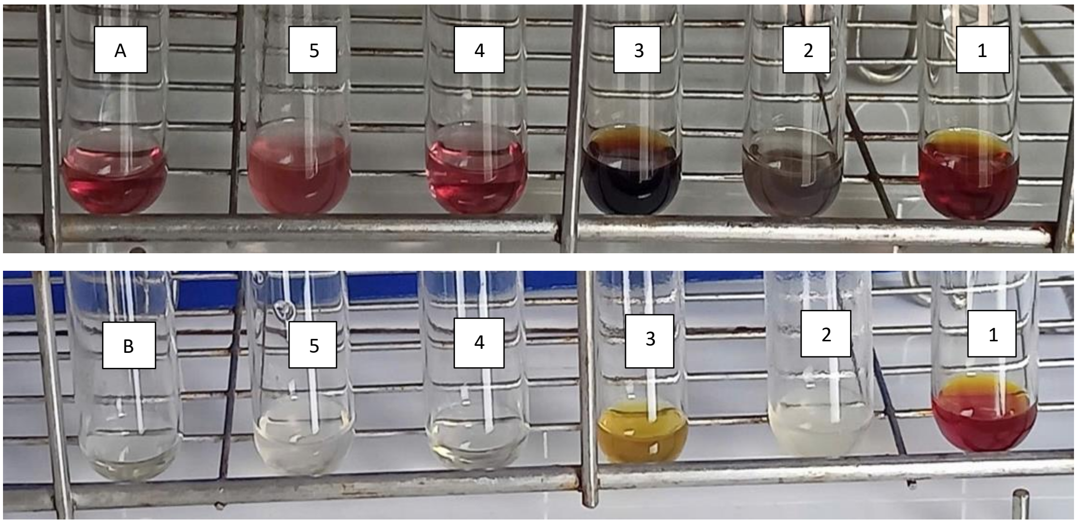

Figure 4. Phytochemical screening in black ginger extracts: (A) rhizomes and (B) roots before reaction, qualitative reaction of alkaloids (1), flavonoids (2), polyphenols (3), saponins (4), and tannins (5)

(Source: Author, 2025)

Table 2 showed that alkaloids, flavonoids, polyphenols, and tannins, except saponins, were present in the extract. Similarly, Julianti et al. (2022) and Thuy et al. (2023) recorded the presence of alkaloids, flavonoids, polyphenols, and tannins in the ethanol extracts of black ginger rhizomes except saponins. Also, according to research by Thuy et al. (2023), water and methanol extracts of black ginger contained alkaloids, flavonoids, polyphenols, quinones, and saponins. With chloroform solvent, black ginger rhizome extract contained quinones, phenolic compounds, steroids, terpenoids, carbohydrates, glycosides, coumarins, alkaloids, proteins, and flavonoids (Atom et al., 2022).

3.2. Effect of drying temperature on moisture content of black ginger rhizome and root slices

The black ginger rhizome and root slices were dried from an initial moisture content of 70.47 and 95.77% to less than 5% using different temperatures. The moisture contents in samples were presented in Figure 5.

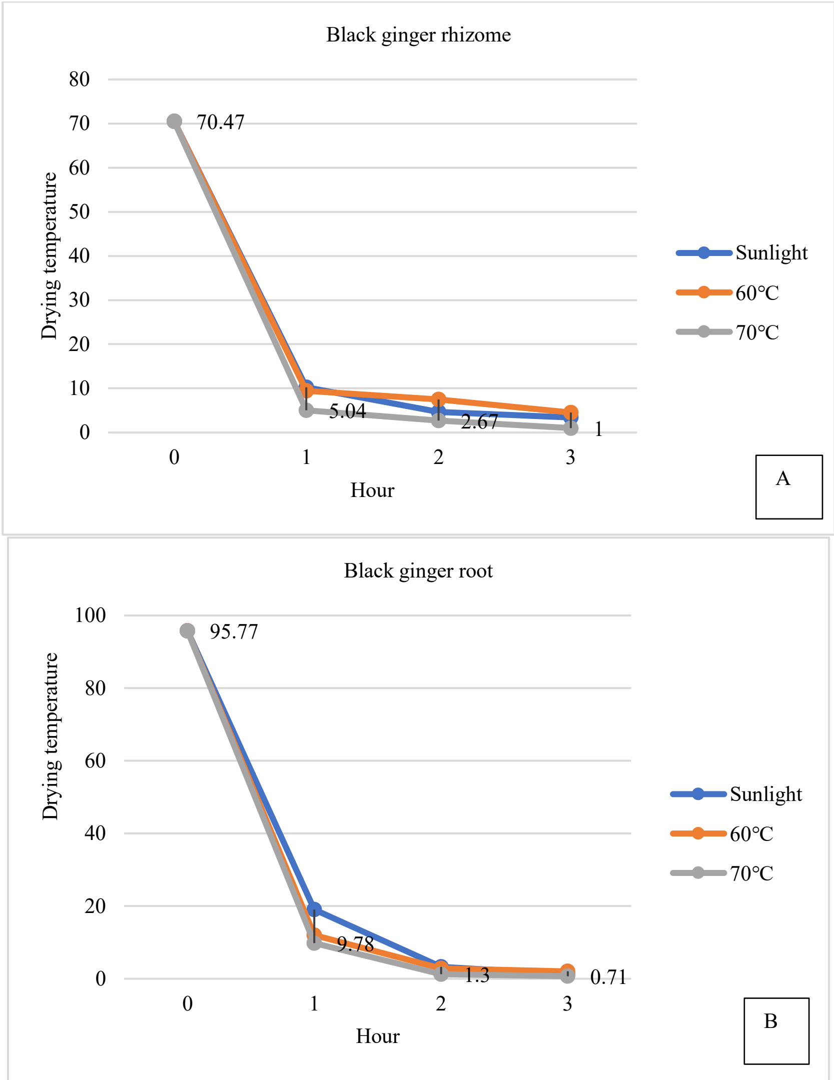

Figure 5. Moisture content of black ginger slices obtained at different drying temperatures: A) Rhizomes, B) Roots

(Source: Author, 2025)

As shown in Figure 5, the drying process occurred quickly in the first hour because the water content in the samples was high, leading to a high rate of water evaporation. In the following hours, the water evaporation occurred slowly because the water remaining in the sample was very small. After 3 hours of drying, the moisture content in all samples decreased below 5%. The sample dried at 70°C had the lowest moisture content in the rhizomes and roots (Table 3). Similarly, Cherrat et al. (2019) also recorded a sharp decrease in the water content in ginger (*Zingiber officinale* Roscoe) slices in the early stages of the drying process due to high water content in the initial sample. Near the end of the drying process, the drying rate decreased, making it more difficult to remove water due to the amount of bound - water to the positions of food components such as protein and starch.

Table 3. Drying time and final moisture content of black ginger slices drying process

Sample	Drying temperature (°C)	Final moisture content (%)	Drying time (Hour)
Black ginger rhizome	Sunlight	3.41 ± 0.57^{a}	3
	60	4.03 ± 0.11^{a}	3
	70	1.00 ± 0.42^{c}	3
Black ginger root	Sunlight	1.24 ± 0.20^{bc}	3
	60	2.03 ± 0.16^{b}	3
	70	0.71 ± 0.29^{c}	3

*According to the LSD test, the means with different letters show a significant difference at the 5% level.

(Source: Author, 2025)

3.3 Effect of drying temperature on total phenolic contents (TPCs) of black ginger rhizome and root extracts

TPCs in black ginger extracts were determined to be equivalent to gallic acid content with the standard curve equation $y = 0.0113x + 0.0463$ ($R^2 = 0.9992$). TPCs in black ginger rhizome and root extracts were significantly different ($P < 0.05$) and were highly related to drying temperature. Table 4 showed that black ginger rhizome samples had much higher TPCs than root samples. The rhizome samples dried at 60°C exhibited the highest TPC value (69.15 mg GAE/g extract) compared to the samples dried at 70°C (68.92 mg GAE/g extract) and exposed to sunlight (66.02 mg GAE/g extract). In contrast, the root sample dried under sunlight had the highest TPC value (13.33 mg GAE/g extract). While, the samples dried at 60 and 70°C had lower TPCs, respectively, 11.17 and 9.85 mg GAE/g extract.

Some research showed that thermal processing was the most important factor affecting the quantity of phenolic substances. Thermal energy can cause the decomposition of cell components, releasing more polyphenols in the dried sample. On the other hand, TPCs can decrease due to the decomposition of phenolic compounds during drying, grinding to powder, and storage (Cherrat et al., 2019). Similar findings were also reported by Muthukumar et al. (2022); drying air temperature at 60°C resulted in optimal TPC and antioxidant activity of black ginger in the investigated temperature range of 40-80°C (Muthukumar et al., 2022). In the same year, Muhammad et al. (2022) recorded that the TPC value in black ginger rhizomes was higher at a drying temperature of 75°C compared to 50°C which was attributed to the release of bound phenolic compounds by the decomposition of cell tissues and to the formation of a new compound in the rhizome. The authors also recorded a drying temperature of 75°C without adversely affecting the postharvest quality of black ginger (Muthukumar et al., 2022). In contrast, a study by Mustafa and Chin (2023) noted that sun drying and ethanol extraction were the most effective methods for preserving and enhancing the quality of ginger (*Zingiber officinale* Roscoe) due to its cost-effectiveness and the efficacy of bioactive compounds.

Table 4. The effect of the dried temperature on TPCs in black ginger extracts

Sample	Drying temperature (°C)	TPCs (mg GAE/g extract)
Black ginger rhizome	Sunlight	66.02 ± 0.27^{b}
	60	69.15 ± 0.77^{a}
	70	68.92 ± 0.57^{a}
Black ginger root	Sunlight	13.33 ± 0.32^{c}
	60	11.17 ± 0.59^{d}
	70	9.85 ± 0.58^{e}

* According to the LSD test, the means with different letters show a significant difference at the 5% level.

(Source: Author, 2025)

Other studies reported similar TPC values on dried black ginger. For example, Tuan and Sanh (2020) recorded the TPC value of black ginger extract as 61,585 mg GAE/g extract with dried black ginger powder at 50°C. Subsequently, Thuy et al. (2023) reported a TPC of 82.06 mg GAE/g extract for ginger dried at room temperature. The difference between these results may be due to the variety, genetics, origin, and part of the black ginger used.

3.4 Effect of drying temperature on antioxidant activity of black ginger extracts

The antioxidant activity of the ethanolic extracts from black ginger was evaluated using the DPPH assay at a concentration ranging from 125 µg/mL to 2000 µg/mL. The results of DPPH free radical scavenging activity are presented in Table 5.

Table 5. DPPH free radical inhibition percentage of the black ginger samples

Concentration (µg/mL)	% Inhibition					
	Rhizome exposed to sunlight	Rhizome dried at 60°C	Rhizome dried at 70°C	Root exposed to sunlight	Root dried at 60°C	Root dried at 70°C
125	8.09 ± 1.85	5.62 ± 0.92	10.26 ± 0.83	8.86 ± 0.44	5.37 ± 0.67	8.67 ± 0.29
250	15.930 ± 0.98	14.79 ± 1.26	20.09 ± 0.53	11.800 ± 0.65	6.95 ± 0.64	13.29 ± 1.01
500	21.35 ± 0.81	24.260 ± 1.04	28.01 ± 0.39	17.05 ± 0.25	12.67 ± 0.81	23.17 ± 0.42
1000	43.64 ± 0.64	42.6 ± 0.67	51.76 ± 0.81	28.23 ± 0.42	18.93 ± 0.51	37.72 ± 0.14
2000	62.43 ± 0.64	63.02 ± 0.53	67.60 ± 1.59	52.79 ± 0.60	52.81 ± 1.82	52.12 ± 0.36

(Source: Author, 2025)

Among black ginger parts, the rhizome extracts had better antioxidant activity than the root extracts. Compared with ascorbic acid, all samples had lower DPPH inhibition percentages. For IC_{50} determination, a standard curve of DPPH was constructed, in which lower values indicated more potent free radical scavenging activity (Table 6). The present study showed that the IC_{50} values of the studied samples were 42.35 to 67.13 times higher than the IC_{50} value of ascorbic acid. Among them, rhizome samples dried at 70°C had relatively better antioxidant activity than the other samples.

Different drying temperatures did not affect the antioxidant activity of black ginger samples. This may be due to increased reducing sugars and Maillard reaction products that usually react with DPPH (Muhammad et al., 2022). Tuan and Sanh (2020), and Julianti et al. (2022) reported that the IC_{50} value of the extract sample from black ginger stem slices dried at 70°C was 140.67 and 145 times higher than the IC_{50} value of ascorbic acid. Thus, the DPPH free radical scavenging efficiency of black ginger extracts in this study was higher than in some previous studies.

Pearson correlation analysis showed that TPCs and black ginger's antioxidant activity (IC_{50}) had a high negative correlation (r = - 0.9412.). These suggest that TPCs are one of the main bioactive compounds in black ginger and are responsible for DPPH-free radical scavenging activity. In addition, black ginger also contains many flavonoids that participate in this activity (Phung et al., 2021; Julianti et al., 2022).

Table 6. IC_{50} values of extracts for DPPH scavenging activity

Sample	Linear regression equation	IC_{50} (µg/mL)
Ascorbic acid	y = 1.6376x + 1.3201 R^2 = 0.9968	29.73
Rhizome exposed to sunlight	y = 0.0287x + 8.0204 R^2 = 0.9644	1462.70
Rhizome dried at 60°C	y = 0.0296x + 7.1658 R^2 = 0.9627	1452.01
Rhizome dried at 70°C	y = 0.0299x + 12.355 R^2 = 0.934	1259.03
Root exposed to sunlight	y = 0.0234x + 5.6204 R^2 = 0.9992	1896.56
Root dried at 60°C	y = 0.0251x - 0.0967 R^2 = 0.9668	1995.88
Root dried at 70°C	y = 0.023x + 8.9251 R^2 = 0.9535	1785.87

(Source: Author, 2025)

4. Conclusion

This study demonstrated that black ginger (Kaempferia parviflora) is a valuable functional ingredient due to its high polyphenol content and potent antioxidant activity. Among the plant's parts, the rhizome stands out compared to the root, showing significantly higher total phenolic content and antioxidant capacity, making it the preferred material for extraction and product development.

Drying temperature was found to affect the retention of bioactive compounds. The highest total phenolic content (69.15 mg GAE/g extract) was achieved at 60°C, while antioxidant activity remained relatively stable at both 60°C and 70°C (IC_{50} values of 1452.01 and 1259.03 µg/mL, respectively). Therefore, 60°C is considered the optimal drying temperature, as it provides the best balance between preserving phenolic compounds and maintaining antioxidant activity.

These findings provide practical guidance for processing black ginger and highlight its potential as a high-quality natural ingredient, particularly when the rhizome is utilized.

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