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Drying's Impact on Antioxidant Compound Levels in *Curcuma heyneana* Valeton & Zijp. (*Temu Giring*)

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Abstract

One plant in the genus *Curcuma* that exhibits potential as a natural antioxidant is *Curcuma heyneana* Valeton & Zijp., commonly known as *temu giring*. This species contains phenolic and flavonoid compounds. The objective of this research was to quantify the phenolic and flavonoid content, as well as assess the antioxidant activity of fresh and dried rhizomes. The extraction methods employed were infusion and decoction. Phenolic content was measured using the colorimetric method with the Folin-Ciocalteu reagent, while flavonoid content was determined using the $AlCl_3$ reagent. Antioxidant activity was evaluated using the FRAP method. The highest phenolic content was observed in the oven-dried decoction extract (EDOD) at 6.258 mg GAE/g, and the highest total flavonoid content was also found in this EDOD at 2.899 mg QE/g. The most potent antioxidant activity among the test samples was exhibited by this EDOD, with a value of 12.064 mol FeEAC/g. The results indicate that higher drying temperatures correlate with increased phenolic and flavonoid levels in the *temu giring* rhizome, consequently influencing the antioxidant activity in the sample.

Keywords: Decoction; Infusion; FRAP; Drying; simplicial*; Temu giring*.

INTRODUCTION

Antioxidant compounds in the human body have a very positive impact. Many emerging diseases are caused by free radicals, both in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as environmental and lifestyle factors. The presence of free radicals, capable of inducing oxidative stress, has been reported as the cause of several disease conditions, such as cardiovascular disease, diabetes mellitus, respiratory diseases, neurodegenerative disorders, and multiple types of cancer (Phaniendra et al., 2014).

In addition to being used as cooking spices, rhizomes are also used as raw materials for traditional medicines and food supplements (Rajkumari & Sanatombi, 2018). One type of *Curcuma* often found is *temu giring* (*Curcuma heyneana* Valeton & Zijp). However, this plant in Indonesia accounts for less than 5% of raw materials used in traditional medicine production, even though it is included in the group of medicinal plants (Jalil, 2019). This plant contains phenolic compounds and flavonoids, contributing to its antioxidant effects (Manuhara et al., 2022; Yustin & Wijayanti, 2018). One of the phenolic compounds with notable pharmacological activity is curcuminoid (Rathore et al., 2020). Studies have also identified this compound in *temulawak* (*Curcuma xanthorrhiza* Roxb.) (Hadi et al., 2018; Rosidi, 2020). The rhizomes of this species are an alternative source of natural antioxidants due to their phenolic content (Santos-Sánchez et al., 2017). Apart from these compounds, flavonoid compounds also act as antioxidants due to their hydroxyl groups, which bind to the carbon of the aromatic ring, enabling them to capture free radicals.

Rhizomes contain high water content, shortening their shelf life and making them prone to microbial spoilage. The drying process is a vital processing technique aimed at post-harvest preservation and serves as a suitable method for retaining the bioactive compounds in it (Chua et al., 2019). The drying process dramatically influences both the pharmacological effects and the chemical composition of medicinal plants, especially compounds that act as antioxidants. The drying process can also affect the total phenolic and flavonoid content of simplicia, both of which contribute to antioxidant activity. Reported research (An et al., 2016) has shown that drying ginger rhizomes increases total phenolic content (TPC), total flavonoid content (TFC), and gingerol levels, enhancing antioxidant activity. Different drying techniques, both conventional and modern, are commonly used to assess their impact on the quality of dried products. More research is needed on *temu giring*, which is abundant but remains underutilized as a medicinal raw material. It is crucial to compare different drying methods to evaluate their effects on

phytochemical content, including total phenolics, total flavonoids, and antioxidant activity in *temu giring*. Testing of *temu giring* using various drying and extraction methods, both conventional and modern, requires further refinement. In addition, phenolic, flavonoid, and antioxidant content in *temu giring* has never been assessed using a microplate reader. Thus, this study aims to provide insights into optimal drying and extraction methods for obtaining phenolic and flavonoid compounds, as well as assessing antioxidant activity using the FRAP method via microplate reader measurements.

MATERIALS AND METHODS

Collection of Raw Materials

Temu giring rhizomes were harvested in October 2023 from Purwontoro District, Wonogiri Regency, Central Java. The samples were identified at the Biopharmaca Cultivation Conservation Unit, Tropical Biopharmaca Research Center, IPB University, Bogor, under letter number 414/IT3.L.P13/TA.00.03/M/B/2023. Harvested samples were washed with running water to remove adhering soil and then drained. Samples were divided into two groups: fresh samples (EIF = Extract Infusion Fresh, EDF = Extract Decoction Fresh) and dried samples (EIOD = Extract Infusion Oven-drying, EISD = Extract Infusion Sunlight-drying, EIAD = Extract Infusion Air-drying, EDOD = Extract Decoction Oven-drying, EDSD = Extract Decoction Sunlight-drying, EDAD = Extract Decoction Air-drying). As shown in **Figure 1**, 200 g of fresh rhizome was weighed for both infusion and decoction, with two replications for each. The rhizomes were sliced crosswise and extracted. The dried rhizome group was weighed at 200 g and chopped crosswise into 3 mm thick slices. The drying methods used were oven drying at 40 °C, air drying at approximately 31–33 °C, and sun drying covered with a black cloth at 34–39 °C. Two replications were performed for each drying process. Once dried, the rhizomes were sorted, ground using a blender, and stored as simplicia powder in a tightly sealed container.

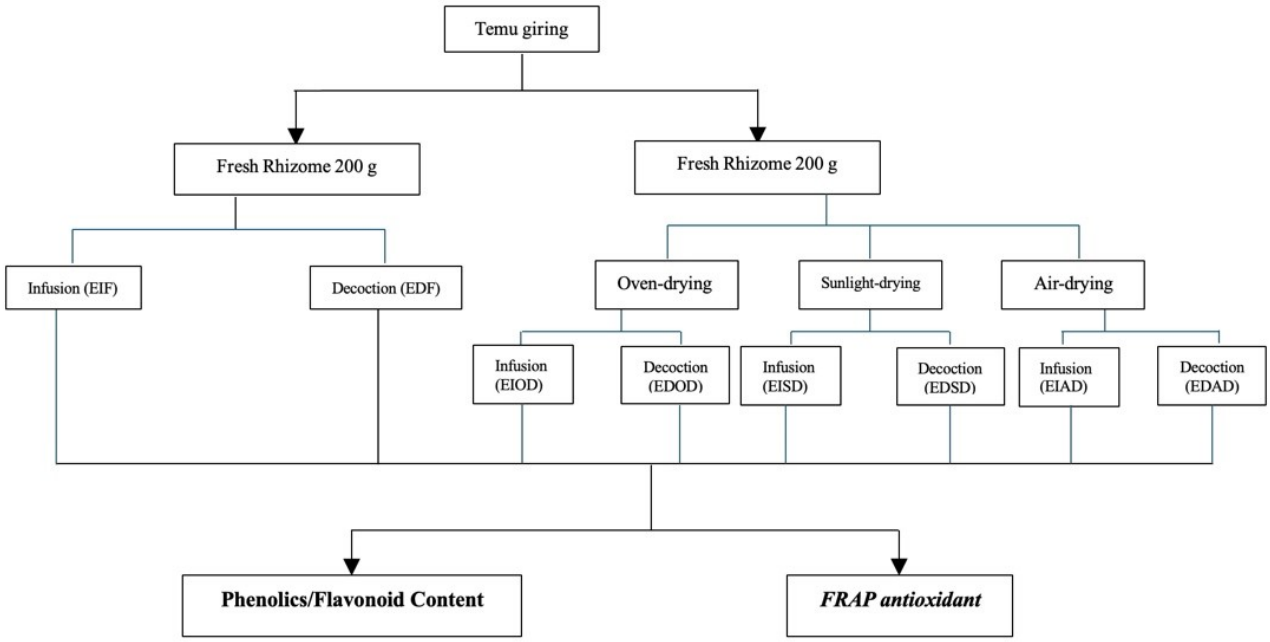

Figure 1. Extraction procedure of temu giring's rhizome fresh and dried material. EIF = Extract Infusion Fresh, EDF = Extract Decoction Fresh, EIOD = Extract Infusion Oven-drying, EDOD = Extract Decoction Oven-drying, EISD = Extract Infusion Sunlight-drying, EDSD = Extract Decoction Sunlight-drying, EIAD = Extract Infusion Air-drying, EDAD = Extract Decoction Air-drying.

Drying Loss Test

Samples of fresh rhizomes and dried powdered rhizomes (2 g each) were placed in a moisture balance container (Mettler Toledo HB43-S). The samples were dried at 105 °C until the instrument automatically stopped. The final sample weight was recorded. All tests were performed in triplicate (Pabenjanan et al., 2024).

Infusion and Decoction Extraction

Fresh or dried *temu giring* rhizomes were mixed with distilled water at a ratio of 1:10. The heating process lasted 15 minutes for infusion and 30 minutes for decoction, starting when the temperature reached 90 °C (as monitored using a thermometer). The solution was stirred occasionally and then filtered. The liquid extract (with a concentration of 10%) was then ready for testing (Ministry of Health Republic of Indonesia, 2017).

Identification of Secondary Metabolites

Phytochemical screening was conducted for the following compounds: flavonoids (Magnesium + HCl), phenolics ($FeCl_3$ 5%), tannins (1% gelatin in 10% NaCl), alkaloids (Mayer, Dragendorff, and Bouchardat reagents), saponins, terpenoids (Salkowski's test), and steroids (Liebermann-Burchard reagents). These tests were conducted on fresh *temu giring* extracts, dried simplicia, and dry *temu giring* extracts (Hanani, 2015).

TLC screening

The identified secondary metabolites were phenolics (curcumin) and flavonoids (quercetin). The stationary phase used was a 60 F254 silica gel plate (Merck, Germany). The mobile phase for phenolics consisted of chloroform: methanol (95:5) with ten drops of acetic acid, and $FeCl_3$ 5% was used as a spray reagent (Ministry of Health Republic of Indonesia, 2017). The mobile phase for flavonoids consisted of chloroform: acetone: formic acid (10:1:1), and spots were visualized using 10% $AlCl_3$. Extracted samples were prepared at 10% concentration, while standards (curcumin from Merck and quercetin from Sigma-Aldrich) were prepared at 0.1% concentration. The sample volume was 10 µL, and the standard volume was 2 µL. Spots were observed under UV 254 nm and 366 nm, and after spraying, under visible light.

Determination of total phenolic and flavonoid content

Total phenolic content was determined based on (Hikmawanti et al., 2023), with slight modifications. Gallic acid (Sigma-Aldrich) was prepared at concentrations of 25, 50, 100, 200, and 400 µg/mL in methanol. Extract and standard solutions (20 µL) were reacted with 100 µL of Folin-Ciocalteu reagent (1:10) for 4 minutes. Then, 75 µL of 7.5% Na_2CO_3 was added, and the mixture was incubated for 120 minutes at room temperature. Absorbance was measured at 750 nm using a Universal Microplate Reader (Bio-Rad® iMark™). All tests were performed in triplicate. Total phenolic content was expressed in mg gallic acid equivalents per gram (mg GAE/g).

Total flavonoid content was determined following (Hikmawanti et al., 2023), with slight modifications. Quercetin (Sigma-Aldrich) was prepared at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL in methanol. Extracts and standards (50 µL) were reacted with 10 µL of 10% $AlCl_3$, 150 µL of methanol, and 10 µL of 1 M sodium acetate. The solution was homogenized and incubated for 40 minutes. Absorbance was measured at 415 nm using a Universal Microplate Reader (Bio-Rad® iMark™). The assay was performed in triplicate. Total flavonoid content was expressed in mg quercetin equivalents per gram (mg QE/g).

FRAP antioxidant

The FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$, mixed in a ratio of 10:1:1 (v/v) (Yumita et al., 2025). Ammonium ferrous sulfate (AFS) was prepared at concentrations of 75, 150, 300, 600, and 1200 µM in distilled water. Test samples (30 µL of test samples, including positive controls such as quercetin, gallic acid, and vitamin C), and FRAP reagent (270 µL) were mixed and incubated at 37 °C in the dark for 30 minutes. The absorbance at 595 nm was then measured using a Universal Microplate Reader (Bio-Rad® iMark™). Testing was conducted in triplicate. The antioxidant activity determined using the FRAP method was expressed as the equivalent value of ferrous ions (Fe^{2+}) with the following formula:

$$FeEAC = \frac{\Delta A}{GRAD} x \frac{Av}{Spv} x\, D\, x\, \frac{1}{Cext}\, x\, 10^5$$

Where FeEAC = Equivalence between antioxidant activity and ferric ion (mol/g); ΔA = Absorbance of sample (after blank correction); GRAD = Calibration curve on AFS; Av = Total volume for the test (300 µL); Spv = Sample volume (30 µL); D = Dilution factor for sample before analysis; Cext = Concentration of sample stock (g/L).

Analysis Data

The obtained data were processed using Microsoft Excel 365 Ver. 16 (2024) to generate a linear regression curve.

RESULTS AND DISCUSSION

Fresh *simplicia* exhibits a bitter taste, a distinctive odor, and a bright yellow color. *Simplicia* dried in an oven or under the sun produces a brownish-yellow color, while *simplicia* dried in the air shows a yellow color. These results align with the organoleptic description of *temu giring*, as listed in the Indonesian Herbal Pharmacopoeia (Ministry of Health Republic of Indonesia, 2017). The drying loss results showed that fresh rhizome samples had a moisture content of 84.81% ±0.25, while drying in the oven resulted in 7.10% ±0.07, air drying in 8.13% ± 0.14, and sun drying in 6.69% ±0.32. According to the Indonesian Herbal Pharmacopoeia (Ministry of Health Republic of Indonesia, 2017), the drying loss rate for *temu giring simplicia* should be <10%, confirming that the *simplicia* of *temu giring*, even when dried using different methods, still meet this standard.

Phytochemical screening results showed that fresh and dried rhizomes, when tested using different methods, contained the same secondary metabolites. The test results are shown in Table 1. Secondary metabolite screening confirmed the presence of phenolics and flavonoids, followed by thin-layer chromatography (TLC) testing. Most *Curcuma* species contain bioactive curcuminoid compounds (Burapan et al., 2020). The curcumin standard consists of 84.0% curcumin, 2.0% bisdemethoxycurcumin, and 13.9% demethoxycurcumin.

This TLC test was conducted to analyze the curcuminoid profiles in fresh and dried *simplicia* decoction and infusion samples. In Figure 2, TLC results after elution and UV254 lamp observation indicate that fresh rhizome infusion and decoction samples contain spots parallel to the comparator (Rf_5 = 0.48). However, under UV366 light and after $FeCl_3$ 5% spraying, no spots were observed parallel to the curcuminoid comparator. In contrast, samples from infusion and decoction dried using three different methods displayed spots that matched the comparator when observed under UV366 (Rf_3 = 0.61, Rf_5 = 0.49, Rf_7 = 0.38). However, after $FeCl_3$ 5% spraying, only one stain spot (Rf_2 = 0.49) corresponded to a curcuminoid compound, namely demethoxycurcumin. The first spot (Rf = 0.61) corresponds to curcumin, the second spot (Rf = 0.49) corresponds to demethoxycurcumin, and the third spot (Rf = 0.38) corresponds to bisdemethoxycurcumin.

Table 1. Phytochemical Screening Results of Simplicia Powder and Liquid Extract of Fresh and Dried Temu Giring Rhizomes.

Sample	Secondary metabolite content						
	Phenolic	Flavonoids	Tannin	Alkaloids	Saponins	Terpenoids	Steroids
EIF	+	+	+	+	+	+	-
EDF	+	+	+	+	+	+	-
EIOD	+	+	+	+	+	+	-
EISD	+	+	+	+	+	+	-
EIAD	+	+	+	+	+	+	-
EDOD	+	+	+	+	+	+	-
EDSD	+	+	+	+	+	+	-
EDAD	+	+	+	+	+	+	-
ODSP	+	+	+	+	+	+	-
SDSP	+	+	+	+	+	+	-
ADSP	+	+	+	+	+	+	-

(+) *indicates that the identified compound is positive,* (-) *indicates that the identified compound is negative*
EIF = Extract Infusion Fresh, EDF = Extract Decoction Fresh and EIOD = Extract Infusion Oven-drying, EISD = Extract Infusion Sunlight-drying, EIAD = Extract Infusion Air-drying, EDOD = Extract Decoction Oven-drying, EDSD = Extract Decoction Sunlight-drying, EDAD = Extract Decoction Air-drying. ODSP = Oven-dried simplicia powder, SDSP = Sunlight-drying simplicia powder, ADSP =Air-drying simplicia powder.

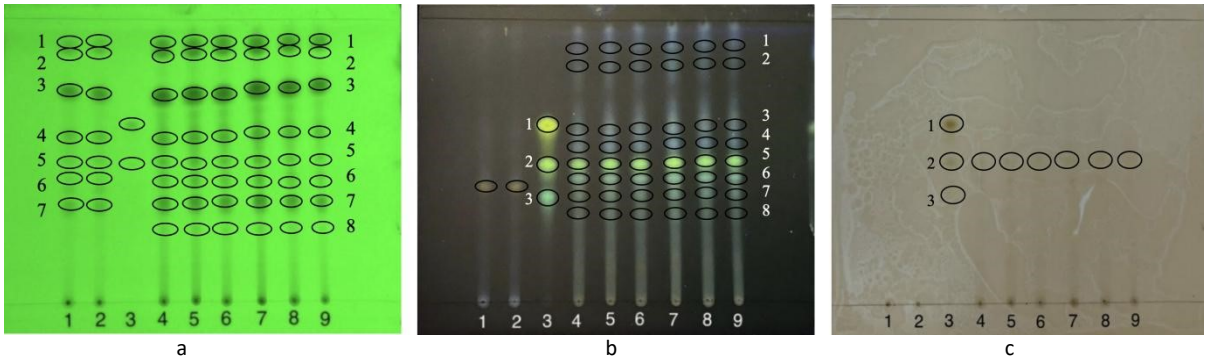

Figure 2. TLC Profile of Curcuminoid Compounds in Fresh and Dried Temu Giring Extracts. (1) EIF = Extract Infusion Fresh, (2) EDF = Extract Decoction Fresh, (3) Curcuminoid, (4) EIOD = Extract Infusion Oven-drying, (5) EIAD = Extract Infusion Air-drying, (6) EISD = Extract Infusion Sunlight-drying, (7) EDOD = Extract Decoction Oven-drying, (8) EDAD = Extract Decoction Air-drying, (9) EDSD = Extract Decoction Sunlight-drying, (a) UV light 254, (b) UV light 366, (c) Visible light after being sprayed with $FeCl_3$ 5%.

Apart from that, quercetin was also used as a standard in TLC screening. This compound has been widely reported for its antioxidant activity. TLC results, observed under UV254 and UV366 lamps and after spraying with 10% $AlCl_3$, showed that fresh and dry extract samples contained spots parallel to the quercetin comparator, with an Rf value of 0.25. After being sprayed with 10% $AlCl_3$, a yellow complex was formed due to the reaction between $AlCl_3$ and the ketone group at C4 and the hydroxyl group (-OH) at the C3 position in flavone or flavonol compounds (Geissman, 1962). Additionally, this yellow stain spot results from forming a complex compound of catechol groups oxidized with aluminum metal in the 10% $AlCl_3$ spray reagent. Based on the chromatogram profile results in Figure 3, it can be concluded that the liquid extract of fresh and dried *temu giring* rhizomes contains quercetin.

The Folin-Ciocalteu colorimetric method works by oxidizing phenolics (alkali salts) or phenolic-hydroxyl groups and reducing heteropoly acids (phosphomolybdic-phosphotungstic) to form molybdenum-tungsten complexes. High phenolic concentrations increase phenolic ions, decreasing heteropoly acid (phosphomolybdic-phosphotungstate) to a molybdenum-

tungsten complex with a solid blue color. Adding Na_2CO_3 makes the solution alkaline, allowing protons to dissociate into phenolic ions. Under these conditions, phenolic compounds react with Folin-Ciocalteu reagent (Yumita et al., 2023). In Table 2, phenolic levels in the extract showed that dried *temu giring* rhizomes had higher total phenolic levels than fresh rhizomes, regardless of the drying method used. According to Prathapan et al. (Prathapan et al., 2009) , phenolic content increases significantly after heating during drying, with levels rising gradually as rhizomes are heated. Oven-dried *simplicia* exhibited the highest phenolic content, whether extracted by infusion (4.728 ± 0.154 mg GAE/g) or decoction (6.258 ± 0.049 mg GAE/g). The phenolic content of EDOD (Extract Decoction Oven-drying) was higher than EIOD (Extract Infusion Oven-drying) because longer heating times enhance compound extraction. The higher dissolved active compound levels result from cell wall decomposition, allowing better solution penetration (Khoddami et al., 2013; Mahmudati et al., 2020).

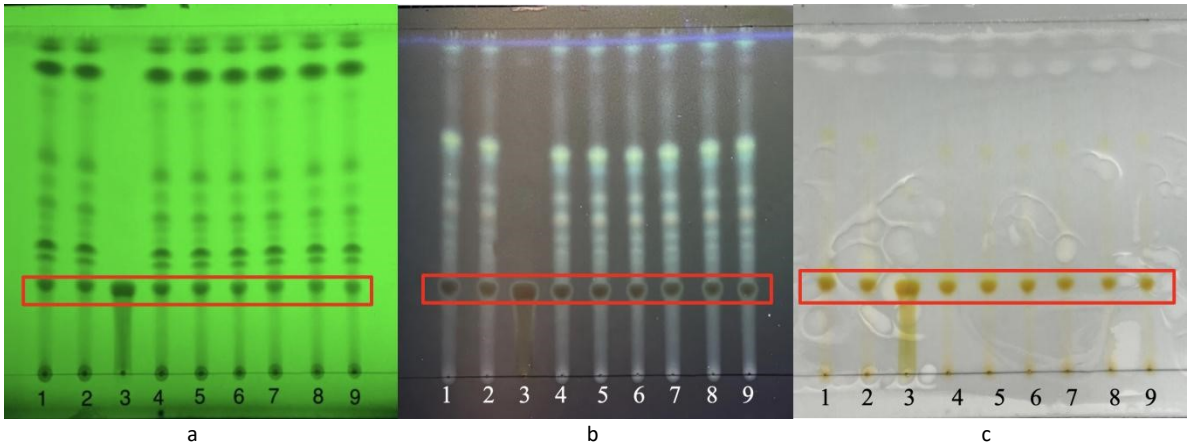

Figure 3. TLC Profile of The Quercetin Compound in Fresh and Dried Temu Giring Extract. (1) EIF = Extract Infusion Fresh, (2) EDF = Extract Decoction Fresh, (3) Quercetin, (4) EIOD = Extract Infusion Oven-drying, (5) EIAD = Extract Infusion Air-drying, (6) EISD = Extract Infusion Sunlight-drying, (7) EDOD = Extract Decoction Oven-drying, (8) EDAD = Extract Decoction Air-drying, (9) EDSD = Extract Decoction Sunlight-drying, (a) UV light 254, (b) UV light 366, (c) Visible light after being sprayed with $AlCl_3$ 10%.

Determination of flavonoid levels using $AlCl_3$ reagent was based on its reactivity with hydroxyl and keto groups, forming an acid-stable complex, and with ortho-dihydroxyl groups, forming an acid-labile complex. This produces a bright yellow color stable in acidic conditions (Anh et al., 2021; Tran et al., 2023). The standard comparator used was quercetin, where the -OH group binds to Al^{3+} and sodium acetate. Table 2 shows that EDOD (Extract Decoction Oven-drying) had the highest flavonoid levels, measuring 2.899 ± 0.093 mg QE/g.

Table 2. Total Phenolics, Flavonoids and Antioxidants of FRAP Fresh and Dried Temu Giring Extract.

Sample	Total Phenolics (mg GAE/g) + SD	Total Flavonoids (mg QE/g) + SD	FRAP (mol FeEAC/g) + SD
EIF	0.235 ± 0.003	0.031 ± 0.002	4.318 ± 0.071
EDF	0.855 ± 0.045	0.315 ± 0.027	6.724 ± 0.008
EIOD	4.728 ± 0.154	2.029 ± 0.055	7.883 ± 0.083
EIAD	3.765 ± 0.058	0.882 ± 0.131	7.147 ± 0.007
EISD	3.412 ± 0.085	0.779 ± 0.006	5.081 ± 0.009
EDOD	6.258 ± 0.049	2.899 ± 0.093	12.064 ± 0.109
EDAD	6.020 ± 0.005	2.799 ± 0.095	6.776 ± 0.081
EDSD	5.605 ± 0.307	2.223 ± 0.099	5.899 ± 0.016

EIF = Extract Infusion Fresh, EDF = Extract Decoction Fresh, EIOD = Extract Infusion Oven-drying, EIAD = Extract Infusion Air-drying, EISD = Extract Infusion Sunlight-drying, EDOD = Extract Decoction Oven-drying, EDAD = Extract Decoction Air-drying, EDSD = Extract Decoction Sunlight-drying.

The antioxidant test using the FRAP method showed linear data with total phenolic and flavonoid levels. The antioxidant effect of dried *simplicia* samples was higher than that of fresh rhizomes, as humidity decreases and polyphenol oxidase enzymes are inactivated during the drying process (Ghafoor et al., 2020). Quercetin, used as a positive control, exhibited antioxidant activity of 37.923,850 ± 0.114 mol FeEAC/g, while gallic acid had 33.071,730 ± 0.052 mol FeEAC/g, and vitamin C had 1.439,640 ± 0.004 mol FeEAC /g. The best reducing

activity was observed in EDOD (Extract Decoction Oven-drying), with a value of 12.064 ± 0.109 mol FeEAC /g.

Polyphenol and flavonoid compounds play a significant role in determining antioxidant activity (Park et al., 2022). A positive correlation was observed between total phenolic and flavonoid levels and antioxidant activity. The higher the phenolic and flavonoid content, the greater the ability to reduce Fe^{3+} ions to Fe^{2+}. Thus, higher phenolic and flavonoid content enhances antioxidant capacity, making Fe^{3+} to Fe^{2+} reduction more efficient and significant.

CONCLUSIONS

Infusion and decoction extracts from *simplicia* dried using the oven method showed higher levels of total phenolics and flavonoids than those extracted from fresh rhizomes, and *simplicia* dried using sun and air drying. Samples with the highest levels of phenolics and flavonoids also showed the best antioxidant activity, indicating a correlation between the two.

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Competing Interests: The authors declare the absence of any potential conflicts of interest.

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