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Research Article

Total Phenolic, Flavonoids Contents, and Antioxidant Activities in The Stems and Rhizomes of Java Cardamom as Affected by Shading and N Fertilizer Dosages

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Abstract: Java cardamom is an herbal medicinal plant known as the "queen of spices." This research aims to determine the influence of shading and nitrogen fertilizer dose on the total phenolics, flavonoids, and antioxidant activity of Java cardamom stems and rhizomes. The study employed a split-plot design with two factors: the level of shading (0, 25, 50, and 75%) as the main plot and the dosage of nitrogen (N) fertilizer (0, 0.9, and 1.36 g polybag^{-1}) as the subplots. Twelve months after planting, the rhizome and stem dried powder were extracted using the sonication-maceration technique with ethanol as the solvent. The 75% shading affected the more outstanding production of total phenolics (1.65 ± 0.59 mg GAE g^{-1} DW), DPPH antioxidant (4.95 ± 0.50 µmol TEAC g^{-1} DW), and FRAP antioxidant (8.94 ± 2.56 µmol TEAC g^{-1} DW) activities of the rhizomes cultivated with 0 g/polybag N in comparison to the stems of the plants. Contrary to phenolics and antioxidant activities, total flavonoids cultivated at 0% shading with 1.36 g polybag^{-1} N of the stems increased concentration than the rhizomes. The results indicated that the 75% shading affected the Java cardamom rhizome's phenolic content and antioxidant activities.

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1. Introduction

Cardamom is a tropical and subtropical plant from the Zingiberaceae family and is one of the Indonesia's export commodities with the third-highest economic value after saffron and vanilla (Rani et al., 2018). Cardamom is a cooking ingredient known as the 'Queen of spices,' which comes from 3 genera: Elettaria, Amomum, and Aframoum (Silalahi, 2017; Hartady et al., 2020). Cardamom grows and develops in the Asian continent, such as Indonesia, Sri Lanka, Nepal, Tanzania, Guatemala, and India (Garg et al., 2016; Abu-Taweel et al., 2018). Qonita et al. (2018) reported that traditionally Java cardamom is often used as a spice in certain dishes, herbal medicines, health drinks, and aromatherapy. The use of herbal medicine and cooking spices from Java cardamom is related to the secondary metabolite content in the essential oil of cardamom (Silalahi, 2017). Pujiarti and Kusumadewi (2020) reported that the availability of essential oils in cardamom provides many benefits, including anti-

inflammatory, antibacterial, therapeutic, and aromatherapy agents. Phytochemical compounds in cardamom consist of flavonoids, sterols, tannins, starch, terpenoids, proteins, and phenols (Moulai-Hacene et al., 2020).

Java cardamom is a possible source of natural bioactive substances containing secondary metabolites such as polyphenol antioxidants. Light intensity, light quality, and photoperiodism influence the accumulation of nearly all forms of secondary metabolites in plants (Zhang et al., 2021). However, the polyphenol content of each type of plant, both sun, and shade plants, showed different results (Idris et al., 2018). Cardamom plants are C3 plants that require 50% sunlight intensity during the day for optimal growth (Alagupalamuthirsolai et al., 2018). The shading will restrict the amount of light plants receive, resulting in a decrease in air temperature and an increase in humidity, both of which are conducive to the development and growth of cardamom. Bhuiyan et al. (2012) reported that the height growth of turmeric and ginger plants in the shade was reported to be more fertile and stronger than those living in the open. Different shading levels were followed by changes in morphology and physiological characteristics of plants that will affect secondary metabolites such as phenolic compounds (Ghasemzadeh and Ghasemzadeh, 2011). Nitrogen is the primary essential nutrient for plants and is required in relatively significant quantities (Leghari et al., 2016). Nitrogen plays a role in the metabolism of protein and chlorophyll, influences the growth and development of vegetative components, and encourages root growth (Leghari et al., 2016).

Several previous studies on the content of secondary metabolites and the bioactivity of cardamom plants have been reported. Winarsi et al. (2013) explained that cardamom leaf extract could control blood glucose levels, which may be antiatherogenic in diabetic rats. In addition, Asra et al. (2019) have tested the phytochemical and antioxidant activity of cardamom leaf extract showing that cardamom leaf extract contains phenolic compounds, flavonoids, tannins, and saponins and may have antioxidant activity. Tmušic et al. (2021) reported that *Melissa officinalis* L. produced higher total phenolic content under shaded conditions than treatment without shade. Ekawati (2018) also reported the same thing, reporting that her administration had a higher total flavonoid content than the shade of *Talinum triangulare* (Jacq.) Willd).). The effect of shading and nitrogen fertilizer dose on the total phenolic, flavonoid, and antioxidant activities of the stems and rhizomes of Java cardamom has not been established. This study aimed to examine the effect of shading and nitrogen fertilizer dose on the phenolic content, total flavonoids, and antioxidant activity of the stems and rhizomes of the Java cardamom plant. Therefore, this study can provide scientific information regarding enhancing the polyphenols of the Java cardamom plant as an antioxidant herbal medicine.

2. Material and Methods

2.1. Plant material and sample preparation

Java cardamom plants are cultivated in the field of Tropical Biopharmaca Research Center, Bogor Agricultural University, West Java, Indonesia, at a latitude of –6.54713°, east longitude 106.71665°, and an altitude of 141 m above sea level. This study used a split-plot design with two factors, namely providing shade as the main plot with four levels: 0 (control) and 25%, 50%, 75% (treatment). The N fertilizer dosage was a subplot with three levels: 0 (control) and 0.90 g polybag-1, 1.36 g polybag-1 (treatment). Therefore, there were 12 combinations of treatments. The treatment was carried out in 3 replications with the plant spacing 50 cm x 50 cm. Java cardamom plants were treated for seven months and harvested at 12 months. The stems and rhizomes were prepared by first washed and cut into small sizes. Next, the stems and rhizomes were sundried for ± 3 days, then milled using a grinding machine into a dried powder with a size of 100 mesh.

2.2. Sample extraction

Dried powders of the samples of stems and rhizomes were extracted based on modifications by Nurcholis et al. (2021a). Briefly, 2 g of stems and rhizomes dried powder were extracted twice using a 10 mL pro-analytical ethanol solvent in a sonicator (Decon Ultrasorics Ltd., England) at a dark-room temperature for 30 min. The homogenate was centrifuged (Kitman-T24, Tomy Kogyo CO. Ltd., Tokyo) for 15 min at 4℃ at 10000 g. The supernatant was then concentrated using a rotary evaporator

(Hahnvapor HS-2005V, Korea) at 50°C and calibrated to 10 mL. The supernatant with a concentration of 0.2 g mL^{-1} was collected and used to determine total phenolics, flavonoids, and antioxidant activity.

2.3. Total phenolic (TPC) and flavonoid content (TFC)

Total phenolic content (TPC) was calculated using the Folin-Ciocalteu method, as described by Khumaida et al. (2019). In a 96-well microplate, 20 µL of ethanol extract of the sample was mixed sequentially with 120 µL of Folin-Ciocalteu (10%) and incubated for 5 min in the darkroom. Then, 80 µL of 10% Na_2CO_3 solution was added, and the mixture was incubated for 30 min at room temperature in the dark. At a wavelength of 750 nm, the absorbance of each treatment was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH). The TPC was reported as mg equivalent gallic acid per gram of dry weight (mg GAE/g DW).

Total flavonoid content (TFC) was assessed using a modified colorimetric approach employing an aluminum chloride ($AlCl_3$) reagent, as described by Calvindi et al. (2020). A 96-well microplate was filled with 10 µL of ethanol extract, 50 µL of ethanol pro analysis, 10 µL of 10% aluminum chloride, 10 µL of glacial acetic acid, and 120 µL of distilled water. The mixture was then homogenized and incubated for 30 min at room temperature and in the dark. At a wavelength of 415 nm, the absorbance of each treatment was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH). TFC was reported as mg equivalent quercetin per gram of dry weight (mg QE g^{-1} DW).

2.4. Antioxidant analysis

Two *in-vitro* methods were used to measure antioxidant activity. The free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Nurcholis et al., 2016), while the reducing power antioxidant was evaluated using the ferric reducing antioxidant power (FRAP) procedure (Calvindi et al., 2020).

DPPH antioxidant activity was determined using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) based on Nurcholis et al. (2016) with a slight modification. A 96-well microplate was loaded with 100 µL of ethanol extract and 100 µL of 125 µM DPPH solution (in ethanol pro analysis). Afterward, the mixture was homogenized and incubated for 30 min in the dark. Finally, the nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) measured the absorbance at 515 nm. The DPPH result is expressed in µmol of Trolox equivalent antioxidant capacity per g of dry weight (µmol TEAC g^{-1} DW).

Analysis of antioxidant activity using the FRAP method was determined using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) according to Calvindi et al. (2021) with a modification. In a row, 10 µL of ethanol extract samples were added with 300 µL of FRAP reagent (made by mixing acetate buffer pH 3.6 with 10 µM TPTZ solution (in 40 µM HCl) and 20 µM $FeCl_3$ (in distilled water) in a v/v/v ratio 10:1:1) was put into microplate-96 wells. Afterward, the mixture was homogenized and left to sit at room temperature for 30 min. At a wavelength of 593 nm, a nano-spectrophotometer was used to detect the absorbance of each sample. The final unit is measured in µmol of Trolox equivalent antioxidant capacity per g of dry weight (µmol TEAC g^{-1} DW).

2.5. Data analysis

By utilizing the IBM SPSS 25 statistical tool, we analyzed the variance (ANOVA). In addition, Tukey's range test was used to compare the data.

3. Results

3.1. Total phenolic content (TPC)

Applying shading levels and nitrogen fertilizer dosages were intended to increase the phenolic component content of the ethanol extract of the stems and rhizomes of Java cardamom. The total phenolics were determined using the Folin-Ciocalteu technique with gallic acid as the phenolic compound standard. The primary group of secondary metabolites in plants that play a significant role in antioxidant activity is phenolics (Rahman et al., 2021). Table 1 depicts the total phenolics of the ethanol extract of the stems and rhizomes.

Table 1. Total phenolic content of ethanol extract of Java cardamom stems and rhizomes

Part of plant	Shade	Total fenolik Content (mg GAE g^{-1} DW)		
		Fertilizer Dosage (g $polybag^{-1}$)		
		0	0.9	1.36
Stems	0%	0.40 ± 0.00bB	0.47 ± 0.04abB	0.68 ± 0.04aA
	25%	0.29 ± 0.04bA	0.37 ± 0.06bA	0.30 ± 0.01aA
	50%	0.62 ± 0.14abA	0.67 ± 0.12abA	0.64 ± 0.05aA
	75%	1.54 ± 0.35aA	1.35 ± 0.30aA	1.30 ± 0.45aA
Rhizomes	0%	0.33 ± 0.00aA	0.41 ± 0.02aA	0.37 ± 0.04aA
	25%	0.66 ± 0.13aA	0.78 ± 0.15aA	0.59 ± 0.05aA
	50%	0.55 ± 0.07aA	1.01 ± 0.32aA	0.95 ± 0.19aA
	75%	1.65 ± 0.59aA	0.70 ± 0.07aA	1.25 ± 0.64aA

Each value is presented as mean ± standard error mean (SEM); numbers followed by different lowercase letters (a-b) showed significant differences ($p<0.05$) in the same column and the same plant parts; Numbers followed by capital letters (A-B) that differ in the same row show significantly different values at ($p<0.05$).

The ethanol extract of Java cardamom rhizomes had a higher total phenolic content in the combination of 75% shading treatment and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$ than in Java cardamom stems ethanol extract. The ethanol extract of Java cardamom stems contains the highest total phenolic content, obtained at 75% shade treatment and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which has 1.54 ± 0.35 mg GAE g^{-1} dry weight. On the other hand, the ethanol extract of stems contains the lowest total phenolic content at 25% shade treatment and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which is 0.29 ± 0.04 mg GAE g^{-1} dry weight. Meanwhile, in the ethanol extract of the Java cardamom rhizomes, the highest total phenolic content was obtained in the 75% shading and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which has 1.65 ± 0.59 mg GAE g^{-1} dry weight. Conversely, the lowest total phenolic content was obtained in the 0% shading and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which has 0.33 ± 0.00 mg GAE g^{-1} dry weight. In this study, it can be seen (Table 1) that each shading level with the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$ did not have a significant effect ($p>0.05$) on the phenolic content of the ethanol extract of the stems and rhizomes of Java cardamom. The combination treatment of 75% shading and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, both in the ethanol extract of stems and rhizomes, obtained the highest total phenolic content compared to other treatments.

3.2. Total flavonoid content (TFC)

The total flavonoid content was evaluated using the aluminum chloride ($AlCl_3$) reagent and quercetin as a standard for flavonoid compounds. The highest total flavonoid content in the ethanol extract of Java cardamom stems and rhizomes can be seen in Table 2.

The ethanol extract of Java cardamom stems had a higher total phenolic content in the combination of 0% treatment and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$ compared to the ethanol extract of Java cardamom rhizomes and other treatments. The ethanol extract of the stems contains the highest total flavonoid content, obtained in the combination treatment of 0% shade (without shade) and the nitrogen fertilizer dosage of 1.36 g polybag-1, which was 0.89 ± 0.13 mg QE g^{-1} dry weight. Conversely, the ethanol extract of the stems contains the lowest total flavonoid content, obtained in the combination treatment of 25% shading with the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which was 0.62 ± 0.06 mg QE g^{-1} dry weight. The ethanol extract of rhizomes contains the highest total flavonoid content, obtained from the combination of 25% shading and the nitrogen fertilizer dosage of 0.9 g $polybag^{-1}$ with a value of 0.47 ± 0.01 mg QE g^{-1} dry weight. In contrast, the ethanol extract of rhizomes contains the lowest total flavonoid content, obtained in the combination treatment of 75% shade and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$ with a value of 0.28 ± 0.01 mg QE g^{-1} dry weight. Based on Table 2, each shading level with the nitrogen fertilizer dosage of 0 g $polybag^{-1}$ shows no significant effect ($p>0.05$) on the total flavonoid content of the ethanol extract of the stems and rhizomes of Java cardamom. TFC showed that the combination of 0% shade treatment and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$ had higher yields than the shading treatment of the ethanol extract

of the stems. In comparison, the ethanol extract of the rhizomes, combination treatment of 25% shading with the nitrogen fertilizer dosage of 0.9 g polybag^{-1} showed a higher total flavonoid content than the treatment without shade with each dosage of nitrogen fertilizer.

Table 2. Total flavonoid content of ethanol extract of Java cardamom stems and rhizomes

Part of plant	Shade	Total flavonoid Content (mg QE g^{-1} DW)		
		Fertilizer Dosage (g polybag^{-1})		
		0	0.9	1.36
Stems	0%	0.76 ± 0.08aA	0.71 ± 0.06aA	0.89 ± 0.13aA
	25%	0.62 ± 0.06aA	0.68 ± 0.04aA	0.69 ± 0.05aA
	50%	0.85 ± 0.11aA	0.80 ± 0.04aA	0.75 ± 0.01aA
	75%	0.75 ± 0.03aA	0.79 ± 0.04aA	0.81 ± 0.01aA
Rhizomes	0%	0.45 ± 0.02aA	0.44 ± 0.06aA	0.45 ± 0.10aA
	25%	0.39 ± 0.06aA	0.47 ± 0.01aA	0.45 ± 0.04aA
	50%	0.42 ± 0.02aA	0.41 ± 0.02aA	0.37 ± 0.05aA
	75%	0.38 ± 0.03aA	0.35 ± 0.03aAB	0.28 ± 0.01aB

Each value is presented as mean ± standard error mean (SEM); numbers followed by different lowercase letters (a-b) showed significant differences (p<0.05) in the same column and the same plant parts; Numbers followed by capital letters (A-B) that differ in the same row show significantly different values at (p<0.05).

The ethanol extract of Java cardamom stems had a higher total phenolic content in the combination of 0% treatment and the nitrogen fertilizer dosage of 1.36 g polybag^{-1} compared to the ethanol extract of Java cardamom rhizomes and other treatments. The ethanol extract of the stems contains the highest total flavonoid content, obtained in the combination treatment of 0% shade (without shade) and the nitrogen fertilizer dosage of 1.36 g polybag^{-1}, which was 0.89 ± 0.13 mg QE g^{-1} dry weight. Conversely, the ethanol extract of the stems contains the lowest total flavonoid content, obtained in the combination treatment of 25% shading with the nitrogen fertilizer dosage of 0 g polybag^{-1}, which was 0.62 ± 0.06 mg QE g^{-1} dry weight. The ethanol extract of rhizomes contains the highest total flavonoid content, obtained from the combination of 25% shading and the nitrogen fertilizer dosage of 0.9 g polybag^{-1} with a value of 0.47 ± 0.01 mg QE g^{-1} dry weight. In contrast, the ethanol extract of rhizomes contains the lowest total flavonoid content, obtained in the combination treatment of 75% shade and the nitrogen fertilizer dosage of 1.36 g polybag^{-1} with a value of 0.28 ± 0.01 mg QE g^{-1} dry weight. Based on Table 2, each shading level with the nitrogen fertilizer dosage of 0 g/polybag shows no significant effect (p>0.05) on the total flavonoid content of the ethanol extract of the stems and rhizomes of Java cardamom. TFC showed that the combination of 0% shade treatment and the nitrogen fertilizer dosage of 1.36 g polybag^{-1} had higher yields than the shading treatment of the ethanol extract of the stems. In comparison, the ethanol extract of the rhizomes, combination treatment of 25% shading with the nitrogen fertilizer dosage of 0.9 g polybag^{-1} showed a higher total flavonoid content than the treatment without shade with each dosage of nitrogen fertilizer.

3.3. Antioxidant activity

The antioxidant activity test was aimed at measuring the total antioxidant capacity of the stems and rhizomes of the Java cardamom plant. The antioxidant activity of stem and rhizome samples was measured using DPPH and FRAP assays, with Trolox as the standard antioxidant compound. The results of the measurement of antioxidant activity using the DPPH and FRAP methods are presented in Table 3 and Table 4, respectively.

Table 3. DPPH antioxidant activity of ethanol extract of Java cardamom stems and rhizomes

Part of plant	Shade	DPPH antioxidant capacity (μmol TEAC g^{-1} DW)		
		Fertilizer Dosage (g $polybag^{-1}$)		
		0	0.9	1.36
Stems	0%	0.20 ± 0.08aB	0.51 ± 0.04aA	0.62 ± 0.01aA
	25%	0.22 ± 0.08aA	0.36 ± 0.17aA	0.23 ± 0.02bA
	50%	0.42 ± 0.17aA	0.48 ± 0.11aA	0.54 ± 0.07aA
	75%	0.61 ± 0.01aA	0.61 ± 0.00aA	0.61 ± 0.00aA
Rhizomes	0%	3.35 ± 0.68aA	3.06 ± 0.04aA	2.32 ± 0.01aA
	25%	3.26 ± 0.38aA	3.58 ± 0.34aA	3.62 ± 0.25aA
	50%	3.36 ± 0.41aA	3.91 ± 0.43aA	4.45 ± 0.30aA
	75%	4.95 ± 0.50aA	3.67 ± 0.38aA	4.03 ± 1.24aA

Each value is presented as mean ± standard error mean (SEM); numbers followed by different lowercase letters (a-b) showed significant differences ($p<0.05$) in the same column and the same plant parts; Numbers followed by capital letters (A-B) that differ in the same row show significantly different values at ($p<0.05$).

Table 4. FRAP antioxidant activity of ethanol extract of Java cardamom stems and rhizomes

Part of plant	Shade	FRAP antioxidant capacity (μmol TEAC g^{-1} DW)		
		Fertilizer Dosage (g $polybag^{-1}$)		
		0	0.9	1.36
Stems	0%	1.91 ± 0.10bA	2.40 ± 0.15aA	3.29 ± 0.62bA
	25%	1.88 ± 0.15bA	2.25 ± 0.32aA	1.76 ± 0.10bA
	50%	2.89 ± 0.57bA	3.12 ± 0.71aA	2.77 ± 0.35bA
	75%	8.75 ± 1.25aA	4.85 ± 0.82aA	8.02 ± 1.66aA
Rhizomes	0%	1.27 ± 0.00bB	1.72 ± 0.03aA	1.32 ± 0.12aAB
	25%	4.35 ± 0.70abA	5.50 ± 1.18aA	4.18 ± 0.22aA
	50%	2.27 ± 0.45abA	5.54 ± 1.42aA	5.84 ± 0.85aA
	75%	8.94 ± 2.56aA	4.28 ± 0.58aA	4.71 ± 1.85aA

Each value is presented as mean ± standard error mean (SEM); numbers followed by different lowercase letters (a-b) showed significant differences ($p<0.05$) in the same column and the same plant parts; Numbers followed by capital letters (A-B) that differ in the same row show significantly different values at ($p<0.05$).

Based on the DPPH antioxidant measurement results (Table 3), the rhizome ethanol extract showed higher antioxidant activity than the stem ethanol extract in the DPPH method. The combination treatment of 0% shading and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$ showed the highest DPPH antioxidant activity compared to other combinations of stem ethanol extract, which was 0.62 ± 0.01 TEAC g^{-1} dry weight. In comparison, the lowest antioxidant activity was shown in a combination treatment of 0% shade and a nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which was 0.20 ± 0.08 mol TEAC g^{-1} dry weight. In the ethanol extract of rhizomes, the combination treatment of 75% shading and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$ showed the highest DPPH antioxidant activity of 4.95 ± 0.50 mol TEAC g^{-1} dry weight. Conversely, the lowest DPPH antioxidant activity was obtained in the combination treatment of 0% shade and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$, which is 2.32 ± 0.01 mol TEAC g^{-1} dry weight. In Table 4, the ethanol extract of the rhizome showed higher FRAP antioxidant activity than the stem ethanol extract. In the ethanol extract of stems, the combination treatment of 75% shade and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$ showed the highest FRAP antioxidant activity, which was 8.75 ± 1.25 mol TEAC g^{-1} dry weight. In comparison, the lowest antioxidant activity resulted from the combination treatment of 25% shading and the nitrogen fertilizer dosage of 1.36 g $polybag^{-1}$, which is 1.76 ± 0.10 mol TEAC g^{-1} dry weight. In the ethanol extract of the rhizome, the highest FRAP antioxidant activity was obtained in a combination of 75% shade treatment and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which was 8.94 ± 2.56 mol TEAC g^{-1} dry weight. Conversely, the lowest FRAP antioxidant activity was obtained in a combination of 0% shade treatment and the nitrogen fertilizer dosage of 0 g $polybag^{-1}$, which is 1.27 ± 0.00 mol TEAC g^{-1} dry weight.

4. Discussion

Phenolic compounds contain one (phenol) or many (polyphenol) phenol rings, notably hydroxy groups that link to aromatic rings to make them easily oxidizable by giving hydrogen atoms to free radicals (Dhurhania and Novianto, 2018). The ability of phenolic compounds to form stable phenoxy radicals in oxidation reactions makes them highly effective antioxidants; Natural phenolic compounds are typically polyphenols that form ether compounds, esters, or glycosides from flavonoids, tocopherols, tannins, coumarins, cinnamic acid derivatives, lignins, and polyfunctional organic acids (Dhurhania and Novianto, 2018). Meanwhile, flavonoids are part of phenolic compounds with a molecular weight. Flavonoid compounds are plants' most prominent family of polyphenolic compounds; more than 6000 flavonoids have been identified (Ghasemzadeh dan Ghasemzadeh, 2011). They are widespread in almost every part of the plant that functions as plant protection from insects and pests, and environmental adaptation (Thakur et al., 2018). Phenolic and flavonoid compounds are secondary metabolites produced by plants in specific quantities under stressed conditions (Kusbiantoro and Purwaningrum, 2018).

The phenolic content of the ethanol extract of the stems and rhizomes of Java cardamom, the combination treatment of 75% shading, and the nitrogen fertilizer dosage of 0 g polybag^{-1}, both in the ethanol extract of stems and rhizomes, give the highest total phenolic content compared to other treatments (Table 1). Busaifi (2017) states that shading conditions will provide stress for plants which can increase secondary metabolite compounds in plants to respond to environmental stress; plants will produce various phytochemicals. The phytochemicals play a vital role in plants' incredible growth and development under stress conditions (Khurshid et al., 2020). In contrast to that reported by Alagupalamuthirsolai et al. (2018), the highest phenolics were obtained in the treatment without shading of the leaf extract of the *E. cardamom* plant. This result is inversely proportional to that obtained in this study; this is due to several factors, namely the type of cardamom used as a sample, climatic factors, growing conditions, physiological conditions, plant age, as well as the cardamom plant parts used in the study, which will also affect the secondary metabolite content in plants. Deepa et al. (2013) reported that the total phenolic content in *A. cardamom* seeds was 1.25 mg GAE g^{-1} (methanol extract) and 0.55 mg GAE g^{-1} (water extract). Tmuši´c et al. (2021) also reported that *Melissa officinalis* L. produced higher total phenolic content under shaded conditions than treatment without shading. Phenolic compounds are secondary metabolites synthesized as a form of defense mechanism for plants (Jain et al., 2017), which are produced in response to changes in UV radiation, temperature, salinity, pathogens, and drought that function as signaling molecules, allelopathic compounds, and detoxifying agents (Karimi et al., 2013).

TFC showed that the combination of 0% shading and the nitrogen fertilizer dosage of 1.36 g polybag^{-1} had higher yields than the shading treatment of the Java cardamom stem ethanol extract. In comparison, for the ethanol extract of the rhizomes, a combined treatment of 25% shade with the nitrogen fertilizer dosage of 0.9 g polybag^{-1} showed a higher total flavonoid content than without shading with each dosage of nitrogen fertilizer. The difference in the total flavonoid content in the stems and rhizomes with shade treatment and fertilizer dosage is because flavonoids are the phenolics most easily produced in epidermal plant cells exposed to high light intensity, which act as a protective response against oxidative damage. However, Karimi et al. (2013) reported that the content of flavonoids and other secondary metabolites in plants are not evenly distributed throughout the plant tissue because the concentration and distribution of secondary metabolites are affected by genetics and environmental factors such as light, moisture, and soil fertility. Nurcholis et al. (2021a) reported that the total flavonoid content in *A. compactum* fruit ranged from 0.19 to 2.26 mg QE g^{-1} DW, indicating that cardamom fruit without shading treatment has higher total flavonoids because environmental light conditions give more effect on flavonoid accumulation. However, Ghasemzadeh and Ghasemzadeh (2011) reported that 60% of shading affected the flavonoid content in *Zingiber officinale* R. plant leaf extract compared to treatment without shade. Solar radiation will increase the accumulation of flavonoids in fruit plants but will reduce the accumulation of flavonoids in *Heliophytes* species and some medicinal plants give the highest total flavonoids in the rhizome sample under shading treatment (Idris et al., 2018). Mir et al. (2017) the low light intensity state showed higher flavonoid productivity (2.5 mg g^{-1} dry weight) when compared to the high sunlight intensity condition in *A. amygdalina* plants. However, Yolci and Tunçtürk (2022) reported was the total content of flavonoids and antioxidant activity, with the best results obtained in the NP0 treatment (without nitrogen and phosphorus fertilizers) on safflower (*Carthamus tinctorius*

L.). This is due to differences in plant species, geography, and environmental factors that cause differences in total flavonoid content and antioxidant activity.

Antioxidants suppress oxidation reactions caused by free radicals, which can cause degenerative diseases by damaging cell wall membranes, unsaturated fatty acids, alkaline DNA, blood vessels, and tissue lipids (Ramadhan et al., 2020). Free radicals are atoms or molecules with unpaired electrons, including superoxide (O_2), hydroxyl (OH^-), peroxyl (RO_2), and hydroperoxyl (HO_2) (Halliwell and Gutteridge, 2015; Nurcholis et al., 2017). Internal enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), tocopherol, and beta-carotene provide the body with antioxidants. The measurement results show that the overall antioxidant activity of FRAP is higher than that of DPPH; this is related to the different reaction mechanisms that occur in the ethanol extract of stems and rhizomes, which are more dominant using the single electron transfer (SET) mechanism than hydrogen atom transfer (HAT). Nurcholis et al. (2021b) reported that the antioxidant activity value of the FRAP method was higher than the DPPH method on cardamom fruit water extract. In addition, Nurcholis et al. (2021a) also reported that the antioxidant activity value of the DPPH method was lower than that of the CUPRAC method. The value of antioxidant activity varies between plant treatments, indicating that the content of compounds that act as antioxidants varies (Zhang et al., 2018). Özyürek et al. (2011) reported that the DPPH method provides the lowest antioxidant activity value compared to other methods because the reagent can influence the stability of DPPH radicals, so high and low antioxidant activity of DPPH is also influenced by several factors, namely the reagents that can be damaged when exposed to light, oxygen, high temperatures, and drying.

Plants can synthesize non-enzymatic antioxidants; however, under stress conditions caused by biotic and abiotic factors, the formation of reactive oxygen species (ROS) rises in plants, resulting in the induction of oxidative stress. In response to increased oxidative stress, plants produce and accumulate more antioxidants (Kasote et al., 2015). Zaini et al. (2021) reported that the antioxidant activity of kencur rhizomes was higher in the 25% shade than in the 50% shade. However, Khusni et al. (2018) reported that the provision of 70% shade was the best shade for producing the highest antioxidant activity compared to other treatments on red spinach (*Alternanthera amoena* Voss.), This difference indicates that the types of plants will give different responses to the effects of both the application and the dosage of nitrogen fertilizer on the secondary metabolite content in plants. Each of the ethanol extracts of the stems and rhizomes of Java cardamom contains flavonoid compounds that act as antioxidants. However, Suhendra et al. (2019) stated that the antioxidant activity value of a sample is not always directly proportional to the total flavonoid content, so there may be other compounds besides phenolic compounds and flavonoids that act as antioxidants. The measurement results show that both cardamom stem and rhizome may act as antioxidants and can be used as candidates for antioxidant herbal medicines. Each part of the plant will produce a different secondary metabolite content based on the function of the plant's organ.

Conclusion

The results presented that the treatment of 75% shade with 0 g polybag^{-1} N improved the phenolic content and antioxidant activity of Java cardamom. The stems contain higher flavonoids than the rhizome part of the plants. Nevertheless, the rhizomes have more potent antioxidant activity than the stems of Java cardamom, as measured by the DPPH and FRAP assays.

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