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Secondary metabolites of Java cardamom leaves extract function under shading and nitrogen doses[1]

Metabólito secundário do extrato de folhas de cardamomo de Java em função de sombreamento e doses de nitrogênio

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HIGHLIGHTS:

The use of 75% shade was recommended to produce the maximum content of phenolics and flavonoids and antioxidant activity.
Without shading, 2,2-diphenyl picrylhydrazyl (DPPH) antioxidant activity was higher.
Compounds 1.8-Cineole and 5-Hydroxy-3,7,4'-trimethoxyflavone were the dominant metabolite extracts of Java cardamom leaves.

ABSTRACT: Java cardamom is a species of herbal medicinal plant endemic to West Java, Indonesia, that has been used for a long time in traditional medicine. The effects of shading and nitrogen doses on the Gas Chromatography-Mass Spectrometry (GC-MS) profile, total phenolic and flavonoid contents, and antioxidant activity in the ethanol extract of Java cardamom leaves were investigated in this study. The experiment was conducted using a split-plot scheme with three replicates. The main plot constituted shading (without, 25, 50, and 75%), and the sub-plot comprised nitrogen doses (control, 0.9, and 1.36 g nitrogen fertilizer per polybag). GC-MS analysis found 27 compounds in the ethanol extract of Java cardamom leaves. The compounds 1.8-Cineole (36.87%) and 5-Hydroxy-3,7,4'-trimethoxyflavone (18.83%) had the highest concentrations in the combination of 25% shade with 0.9 g N fertilizer per polybag and 50% shade with 1.36 g N fertilizer per polybag. The highest total phenolic content (1.26 mg gallic acid equivalent g^{-1} dry weight), flavonoid content (3.42 mg quercetin equivalent g^{-1} dry weight), and ferric reducing antioxidant power (FRAP) antioxidant activity (5.80 µmol Trolox equivalent antioxidant capacity g-1 dry weight) were found with 75% shade. Metabolites 1-Methyl-4-isopropyl-cis-3-hydroxycyclohex-1-ene-6-one, 4-propoxy-catechol, cyclohexane, tert-pentyl-, cis-p-Menth-2,8-dienol, cis-carveol, and cis-p-mentha-1(7),8-dien-2-ol were responsible for antioxidant activity in the ethanol extract of Java cardamom leaves. Shade of 75% is recommended to produce optimal antioxidant activity and phenolic and flavonoid content from Java cardamom leaf extract.

Key words: *Amomum compactum* Soland Ex. Maton, abiotic stress, antioxidant, GC-MS, polyphenols

RESUMO: O cardamomo de Java é uma espécie de planta medicinal herbácea endêmica de Java Ocidental, na Indonésia, que tem sido usada há muito tempo na medicina tradicional. Os efeitos do sombreamento e das doses de nitrogênio no perfil de cromatografia gasosa-espectrometria de massas (GC-MS), teores de fenólicos e flavonoides totais e atividade antioxidante em extrato etanólico de folhas de cardamomo de Java foram investigados neste estudo. O experimento foi conduzido em esquema de parcelas subdivididas com três repetições. A parcela principal foi constituída de sombreamento (sem, 25, 50 e 75%) e as subparcelas foram compostas por doses de nitrogênio (testemunha, 0.9 e 1.36 g de nitrogênio por saco). A análise GC-MS encontrou 27 tipos de compostos no extrato etanólico das folhas de cardamomo de Java. Os compostos 1,8-Cineol (36.87%) e 5-Hidroxi-3,7,4'-trimetoxiflavona (18.83%) apresentaram as maiores concentrações na combinação de 25% de sombreamento com 0.9 g saco $poli^{-1}$ de fertilizante N e 50% sombreamento com 1.36 g saco $poli^{-1}$ de fertilizante N. O maior teor de fenólicos totais (1.26 mg de ácido gálico equivalente g-1 peso seco), flavonóides (3.42 mg de quercetina equivalente g^{-1} peso seco) e atividade antioxidante de poder antioxidante redutor férrico (FRAP) (5.80 µmol Trolox capacidade antioxidante aquivalente g^{-1} peso seco) foram encontrados na sombra de 75%. Verificou-se que 1-Metil-4-isopropil -cis-3-hidroxiciclohex-1-eno-6-ona, 4-propoxi-catecol, ciclohexano, terc-pentil-, cis-p-Menth-2,8-dienol, cis-carveol, dan cis-p-mentha -1(7),8-dien-2-ol foram os metabólitos responsáveis pela atividade antioxidante no extrato etanólico das folhas de cardamomo de Java. O sombreamento de 75% é recomendado para produzir maior teor fenólico e flavonóide e ótima atividade antioxidante do extrato de folhas de cardamomo de Java.

Palavras-chave: *Amomum compactum* Soland Ex. Maton, estresse abiótico, antioxidante, GC-MS, polifenóis

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Introduction

Indonesia has a wealth of biodiversity, of which there are plants that can produce essential oils. Cardamom (*Amomum compactum* Soland Ex. Maton) is an aromatic herbal plant that has been used for a long time as a traditional medicine, has pharmacological activity, and has been cultivated in several Asian, American, and African countries, such as Sri Lanka, India, Nepal, Indonesia, Guatemala, and Tanzania. The cardamom plant is a C3-type plant that requires 50% sunlight intensity during the day for optimal growth (Alagupalamuthirsolai et al., 2018). Shade treatment results in lower sunlight, which increases vegetative growth (Leghari et al., 2016).

Nitrogen is the leading essential food for plants and is needed in relatively large quantities. It plays a role in the formation of protein and chlorophyll, influences the growth and development of vegetative parts, stimulates root growth, and encourages the absorption and utilization of other nutrients, including potassium and phosphorus, thus controlling plant growth (Leghari et al., 2016).

Several previous studies have reported the content of secondary metabolites and bioactivity in cardamom plants. On Java cardamom stem and rhizome extract and *Elettaria cardamomum* Maton leaf extract, Arista et al. (2023a) and Alagupalamuthirsolai et al. (2018), respectively, showed that shading was effective in increasing secondary metabolite content. However, research has yet to be conducted on the effect of shade and nitrogen fertilizer doses on secondary metabolite content, antioxidant activity, and metabolite profiles in Java cardamom leaves.

This research was carried out with the aim of identifying secondary metabolite profiles with Gas Chromatography-Mass Spectrometry (GC-MS) and evaluating the total phenolic content (TPC) and total flavonoid content (TFC) and antioxidant activity (2,2-diphenyl picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP)) of Java cardamom leaves treated with shade and doses of nitrogen fertilizer.

Material and Methods

Java cardamom cultivation was carried out at the Biopharmaca Conservation and Cultivation Unit Garden, Cikabayan Tropical Biopharmaca Study Center, Bogor Agricultural University, West Java, Indonesia, at a latitude of -6.54713°, east longitude 106.71665°, and altitude of 141 m above sea level.

This study used a split-plot design with two factors, with shading (0, 25, 50, and 75%) as the main plot and the doses of N fertilizer (0, 0.9, and 1.36 g per polybag) as subplots, with three repetitions and plant spacing of 50 × 50 cm. Java cardamom plants were grown for 7 months and harvested at 12 months. The leaves were dried in the sun for ± 3 days and then ground using a grinding machine with a 100-mesh screen.

The dried powder of the Java cardamom leaves was extracted using a modified sonication-centrifugation method based on Nurcholis et al. (2021a). Briefly, 2.0 g of dried cardamom leaf powder was extracted twice with 10 mL of ethanol pro-analyst solvent and sonicated using a sonicator (Decon Ultrasonics Ltd., England) in the dark at room temperature for 30 min. The resulting homogenate was then centrifuged for 15 min at 4 °C and a speed of 10,000 × g (Kitman-T24, Tomy Kogyo CO. Ltd., Tokyo). The supernatant resulting from the centrifugation process was then concentrated using a rotary evaporator (Hahnvapor HS-2005V, Korea) at 50 °C and measured up to 10 mL. The concentrated supernatant (0.2 g mL^{-1}) of leaf cardamom was used as the extract to determine TPC, TFC, and antioxidant activity and to perform GC-MS analysis.

TPC was determined using the Folin-Ciocalteu method based on Nurcholis et al. (2022), with modifications. Briefly, 20 µL of ethanol extract from Java cardamom leaves was added to 120 µL of Folin-Ciocalteu reagent (10%) into a 96-well microplate and incubated for 5 min in the dark at room temperature. Then, 80 µL of Na_2CO_3 solution (10%) was added, and it was incubated again for 30 min in the dark at room temperature. The absorbance was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 750 nm. The TPC was expressed as mg gallic acid equivalent per g dry weight (mg GAE g^{-1} DW).

The TFC was determined by calorimetry using an aluminum chloride ($AlCl_3$) reagent based on Nurcholis et al. (2021a). Briefly, 10 µL of Java cardamom leaf extract was added to 50 µL of pro-analyst ethanol, 10 µL of 10% aluminum chloride ($AlCl_3$), glacial acetic acid (CH_3COOH), and 120 µL of distilled water were placed onto a 96-well microplate. They were then homogenized and incubated for 30 min in the dark at room temperature. Absorption was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 415 nm. The TFC was expressed as mg quercetin equivalent per g dry weight (mg QE g^{-1} DW).

Antioxidant activity testing was carried out using two methods: the DPPH and FRAP methods. Using the DPPH method, the antioxidant activity was measured using a nano-spectrophotometer based on Arista et al. (2023a) with modifications. Briefly, 100 µL of ethanol extract from Java cardamom leaves was added to a 96-well microplate with 100 µL of 125 µM DPPH solution (in ethanol pro-analyst). They were then homogenized and incubated in the dark at room temperature for 30 min. Absorbance was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 515 nm. The final unit is expressed in µmol Trolox equivalent antioxidant capacity per g dry weight (µmol TEAC g^{-1} DW).

The analysis of antioxidant activity using the FRAP method was measured with a nano-spectrophotometer based on Arista et al. (2023a) with modifications. Briefly, 10 µL of ethanol extract from Java cardamom leaves was added to 300 µL of FRAP reagent (prepared by mixing acetate buffer (pH 3.6) with 10 µM TPTZ solution (in 40 µM HCl), and 20 µM $FeCl_3$ (in distilled water) with a ratio of v/v/v 10:1:1) in a 96-well microplate. They were then homogenized and incubated at room temperature for 30 min. The absorbance was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 593 nm. The final unit was expressed in µmol trolox equivalent antioxidant capacity per g dry weight (µmol TEAC g^{-1} DW).

The metabolite profiling of Java cardamom leaf extract from each treatment was identified using GC-MS based on Nurcholis et al. (2021b) with modifications. Sample preparation was carried out by mixing 1 mL of each repetition per treatment and homogenizing it for ± 5 min so that 12 samples of Java cardamom leaf extract were obtained with a 0.2 g mL^{-1} concentration. Metabolite profiling analysis was carried out using an Agilent Technologies GC-MS 5977 equipped with an HP-5MS 5% Phenyl Methyl Silox capillary column (internal diameter 30 m x 250 µm, film thickness 0.25 µm, and maximum temperature 325°C) with a carrier gas flow rate (He) of 1 mL min^{-1}. One microliter of the sample was injected into GC-MS at an injection temperature of 290°C. The temperature of the GC-MS was set for operating conditions with an initial temperature of 60°C (held 0 min), then increased at a rate of 10°C min^{-1} until the final temperature of 290°C (held 8 min), with a total time required of 31 min. The mass spectrophotometer was operated at 70 eV, and the spectral range of the scanned mass ranged from 35 to 650 amu. Each sample was analyzed once without repetition. Identification, including the name, chemical structure, and molecular weight of each cardamom essential oil, was calculated based on the relative peak area (percent area) of the chromatogram, with the spectrum of known compounds in the standard library and chemical components confirmed by comparing the retention time (RT) with the Willey 9 database library and adjusted with the data in the PubChem data.

Statistical analysis of TPC, TFC, and antioxidant activity was carried out using analysis of variance based on a split-plot design at $p \leq 0.05$ and Tukey's test using IBM SPSS Statistics 25 software. Regression analysis and correlation between polyphenol content (TPC and TFC) and antioxidant activity (DPPH and FRAP) were applied using GraphPad Prism 8 for Windows (GraphPad Software Inc., San Diego, California, USA), Version 8.0. 1. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) dendogram analysis were performed using Metaboanalyst (Arista et al., 2023b).

Results and Discussion

The TPC and TFC of the ethanol extract of Java cardamom leaves are presented in Table 1.

The variance analysis showed that the TPC of the ethanol extract of Java cardamom leaves was only significantly affected by shade. In contrast, the N fertilizer doses and the interaction showed no significant effects (Table 1). The ethanol extract of Java cardamom leaves had the highest average TPC, namely 1.26 mg GAE g^{-1} DW, at 75% shade (Table 1). The lowest TPC, which was 0.51 mg GAE g^{-1} DW, was obtained with 25% shade. Treatment with 75% shade can potentially obtain the highest TPC in Java cardamom leaves.

The analysis of variance showed that only shading affected the TFC of the ethanol extract of Java cardamom leaves. Based on the measurement results, the highest TFC, namely 3.42 mg QE g^{-1} DW, in the ethanol extract of Java cardamom leaves was obtained with 75% shade. The lowest TFC was obtained with 50% shade (1.67 mg QE g^{-1} DW). The 75% shade treatment showed the highest TPC and TFC content in the ethanol extract of Java cardamom leaves. In comparison, the 25% shade treatment showed the lowest TPC in the ethanol extract of Java cardamom leaves, but the TFC was lowest in the 50% shade treatment.

The highest TPC and TFC in the cardamom leaf extract differed by treatment. This is because the accumulation of bioactive compounds is influenced by environmental factors such as light, temperature, variety origin, vegetation period, harvest period factors, storage conditions, pests/diseases, altitude, and extraction techniques used (Sivakumar et al., 2017). Shaded conditions can cause plants to become stressed; thus, they increase the production of secondary metabolite compounds in response to the environmental stress that occurs. However, different results were reported by Tarfaoui et al. (2022), who reported that the TPC in the ethanol extract of the seeds of *E. cardamomum* (green cardamom) was higher without shade treatment and N fertilizer (33.45±0.35 mg GAE g^{-1} DW). This difference was based on differences in climate, conditions where the plant grew, age, types and parts of the plant used, and technique and type of solvent used in the extraction process, which affected the secondary metabolites extracted in the cardamom plant.

Nurcholis et al. (2022) also reported the methanol extract of Java cardamom seeds using the ultrasonic-assisted extraction (UAE) method without shade treatment and doses of N fertilizer (1.611 mg QE g^{-1} DW). The TFC in this study was higher than in previous studies, which showed that shade affected the TFC of Java cardamom plants. This was also supported by Arista et al. (2023a), who reported that 75% shade affected the phenolic and flavonoid content in the stem

Table 1. Total phenolic content (TPC) and flavonoid content (TFC) of the ethanol extract of Java Cardamom leaves

Treatment	Variables	
	TPC	TFC
Shade (%)		
0	0.58	2.35
25	0.51	1.69
50	0.52	1.67
75	1.26	3.42
Regression	$y = 0.0003*x^2 - 0.0161*x + 0.6125$; $R^2 = 0.95$	$y = 0.001**x^2 - 0.0595*x + 2.4065$; $R^2 = 0.97$
Fertilizer doses (g per polybag)		
0	0.78a	2.42a
0.9	0.66a	2.17a
1.36	0.75a	2.24a
Interaction	ns	ns

Numbers in the same column followed by the same letter are not significantly different by Tukey test (ns = not significant at $p > 0.05$). **, *: Significant at $p \leq 0.01$ and 0.05, respectively, by F-test

and rhizome extract of Java cardamom compared to treatment without shade. During the growth period of the cultivated Java cardamom plants, the weather changes from summer to the rainy season, which can affect the TFC. Soil temperature and low soil moisture also increase the expression of PAL, a key enzyme in synthesizing flavonoids. In addition, plants grown under nitrogen-deficient conditions in an optimum growing environment are thought to contain more secondary metabolites than plants grown in high-nitrogen environments.

Phenolic compounds for plants function in protecting against UV radiation, excess water loss, herbivores, and pathogens, attracting animals in nature to help pollinate and spread seeds, enacting allelopathy, and signaling defense reactions to biotic and abiotic stressors. Flavonoid compounds belong to a sizable phenolic group biosynthesized from acetic acid/phenylalanine derivatives via the shikimate acid pathway (Leghari et al., 2016).

The results of measuring the antioxidant activity of the DPPH method and the FRAP method on the ethanol extract of Java cardamom leaves are presented in Table 2.

The analysis of variance on the DPPH antioxidant activity of ethanol extract of Java cardamom leaves showed a statistically significant effect of the shade treatment. However, based on the average value, the 0% shade treatment showed the highest DPPH antioxidant activity (0.99 µmol TEAC g^{-1} DW). In contrast, the lowest DPPH antioxidant activity was shown in the 75% shade (0.52 µmol TEAC g^{-1} DW).

Based on the data shown (Table 2), the FRAP antioxidant activity in the ethanol extract of Java cardamom leaves was only significantly affected by the shade percentage. The 75% shade treatment had the highest average FRAP antioxidant activity compared to the other treatments (5.80 µmol TEAC g^{-1} DW). In comparison, the lowest FRAP antioxidant activity value was indicated by the 50% shade treatment, (4.26 µmol TEAC g^{-1} DW). The results of measuring the two antioxidant activity methods as a whole showed that FRAP antioxidant activity was higher than DPPH antioxidant activity in the ethanol extract of Java cardamom leaves. This indicates that the ethanol extract of Java cardamom leaves is more dominant using the single electron transfer (SET) mechanism than hydrogen atom transfer (HAT). This is in line with research conducted by Nurcholis et al. (2021c), who reported that the value of the antioxidant activity of the FRAP method was higher than that of the DPPH method in aqueous extracts of cardamom fruit. This is due to the stability of the DPPH radical, which can be affected by the reagent. In addition, the nature of the reagent, which can be damaged when exposed to light, oxygen, high temperature, pH, and drying, affects the level of DPPH antioxidant activity in an extract of the test sample (Danet, 2021).

Based on the results of measurements of antioxidant activity, the 0% shade treatment had the highest DPPH antioxidant activity in the ethanol extract of Java cardamom leaves, in contrast to the results of measuring the FRAP antioxidant activity, which showed that the 75% shade had higher antioxidant activity than other treatment combinations. However, overall, the antioxidant activity using the FRAP method with 75% shade was higher than that of the DPPH method. Suhendra et al. (2019) stated that the value of the antioxidant activity of a sample is not always directly proportional to the TFC; thus, it is suspected that there are other compounds besides phenolic and flavonoid compounds that act as antioxidants.

The Pearson correlation value (r) between TPC and DPPH antioxidant activity in each treatment combination of ethanol extract of Java cardamom leaves showed a negative correlation (r = -0.3676), which had a weak correlation relationship because the resulting coefficient value was less than 0.5 ($p \leq 0.05$) (Figure 1A).

Meanwhile, the results of the Pearson correlation test between TPC and the value of FRAP antioxidant activity in each treatment combination of ethanol extract of Java cardamom leaves showed a positive correlation (r = 0.8704), with the correlation coefficient value indicating a strong, significant, and unidirectional correlation ($p \leq 0.05$) (Figure 1B).

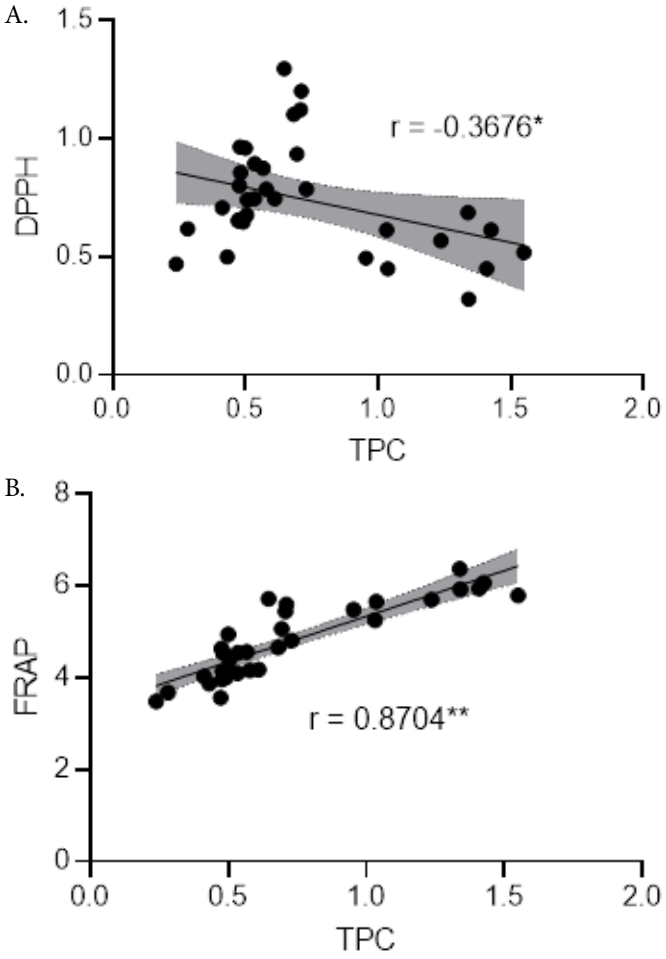
This indicates that the FRAP antioxidant capacity in each treatment combination of the ethanol extract of Java cardamom leaves is more strongly influenced by phenolic compounds with a correlation value close to 1 compared to the correlation value of antioxidant activity and DPPH, which means that the phenolic compounds contained in the ethanol extract of Java cardamom leaves can act as compounds. This aligns with research conducted by Muflihah et al. (2021), who reported that antioxidant activity and TPC in C. longa plants had a significant correlation.

The Pearson correlation value (r) between TFC and DPPH antioxidant activity was not significant (Figure 2A).

Table 2. Antioxidant activity of DPPH and FRAP methods of ethanol extract of Java cardamom leaves

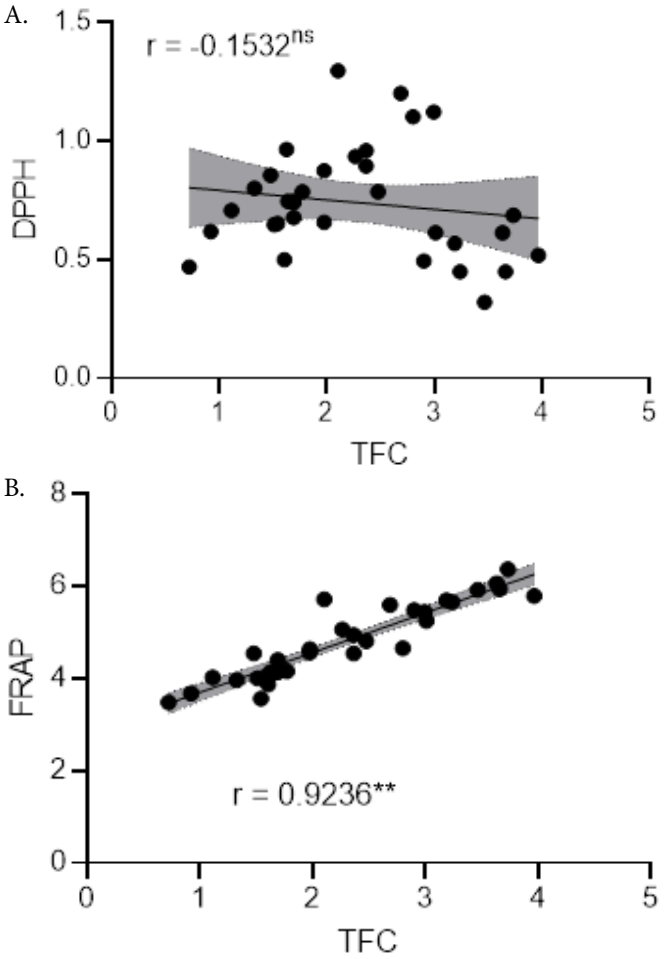
Treatment	Variables	
	DPPH	FRAP
Shade (%)		
0	0.99	4.71
25	0.80	4.40
50	0.73	4.26
75	0.52	5.80
Regression	$y = -0.0059^{**}x + 0.982$; $R^2 = 0.97$	$y = 0.0007^{*}x^2 - 0.043^{*}x + 4.7855$; $R^2 = 0.92$
Fertilizer doses (g per polybag)		
0	0.78a	4.98a
0.9	0.72a	4.75a
1.36	0.72a	4.67a
Interaction	ns	ns

Numbers in the same column followed by the same letter are not significantly different by Tukey test (ns = not significant at $p > 0.05$). **, *: Significant at $p \leq 0.01$ and 0.05, respectively, by F test



*; **- Significant at p ≤ 0.05 by t test

Figure 1. Scatter plot of Pearson correlation between (A) total phenolic content of the ethanolic extract of Java cardamom leaves and the DPPH antioxidant activity; (B) total phenolic content of the ethanol extract of Java cardamom leaves and the FRAP antioxidant activity



** - Significant at p ≤ 0.05 by t test; ns- not Significant at p > 0.05 by t-test

Figure 2. Scatter plot of Pearson correlation between (A) total flavonoid content of the ethanolic extract of Java cardamom leaves and the DPPH antioxidant activity; (B) total flavonoid content of the ethanol extract of Java cardamom leaves and the FRAP antioxidant activity

Meanwhile, the Pearson correlation test between TFC and FRAP antioxidant activity showed a strong positive correlation (r = 0.9236) (Figure 2B).

A positive and significant correlation was also found between TFC and CUPRAC antioxidant activity in the ethanol extract of Java cardamom fruit (Nurcholis et al., 2021a). This also shows that flavonoid compounds also act as antioxidants in the ethanol extract of Java cardamom leaves.

The ethanol extract of Java cardamom leaves with a combination of shade level, and nitrogen fertilizer doses was analyzed for its compound content using the GC-MS instrument. Twenty-seven metabolites were identified in 12 treatment combinations of ethanol extract from Java cardamom leaves. Chemical compounds were identified based on RT, peak area, and molecular formula (Table 3). The peak width was set at 0.1 with the initial threshold set at 22, so that of the 12 treatment combinations, the identified compounds could be categorized into different compound groups (Figure 3), namely monoterpenes (15), diterpenes (3), sesquiterpenes (1), alkyl (1), phenol (1), ketone (1), fatty acid amides (1), fatty acids (2), alkaloids (1), and flavonols (1). Monoterpene compounds were the dominant class in the ethanol extract of Java cardamom leaves in the 12 treatment combinations: T1 (67%), T2 (58%), T3 (69%), T4 (58%), T5 (64%), T6 (64%), T7 (67%), T8 (55%), T9 (60%), T10 (50%), T11 (53%), and T12 (52%). Overall, monoterpenes (56%) had higher concentrations than sesquiterpenes (4%) in the ethanol extract of Java cardamom leaves in the 12 treatment combinations (Figure 3). Similarly, Arista et al. (2023a) reported that the concentration of monoterpene compounds (70%) was higher than sesquiterpene compounds (19%) in four parts of Java cardamom essential oil. This difference is based on the effect of treatment, source of raw materials, and growth location on the amount and quality of plant metabolites. The details of each identified compound are presented in Table 3. The ethanol extract of Java cardamom leaves treated with a combination of shade level and nitrogen fertilizer dose was analyzed for compound content using the GC-MS instrument.

In detail, the components of the compounds identified from the 12 treatment combinations of the ethanol extract of Java cardamom leaves each consisted of T1 (12), T2 (12), T3 (16), T4 (12), T5 (11), T6 (11), T7 (12), T8 (11), T9 (10), T10 (16), T11 (17), and T12 (21) (for an explanation of parameter symbols, see Table 3), representing 83.64, 86.83, 88.52, 90.91, 81.27, 91.74, 78.4, 78.21, 81.18, 77.89, 77.66, and 70.96% of the total volatile compounds, respectively.. The results of the

Table 3. Volatile compounds identified in 12 ethanol extracts from Java cardamom leaves

No	Compound	Compound group	MF	MW (g mol^{-1})	RT (min)	% Area											
						T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
1	p-Cymene	Monoterpene	$C_{10}H_{14}$	134.22	4.811									2.57			0.91
2	o-Cymene	Monoterpene	$C_{10}H_{14}$	134.22	4.790	2.99	2.96	2.48	4.41	3.30	4.37	2.33					
3	1,8-Cineole	Monoterpene	$C_{10}H_{18}O$	154.25	4.915	23.49	22.88	19.53	33.35	36.87	35,.5	18.19	19.38	22.97	15.88	14.86	12.69
4	*trans*-p-Mentha-2,8-dienol	Monoterpene	$C_{10}H_{16}O$	152.23	6.083	3.64	3.46	3.32	4.53	4.32	4.35	3.92	5.10	4.60	3.69	3.93	3.80
5	*cis*-p-Menth-2,8-dienol	Monoterpene	$C_{10}H_{16}O$	152.23	6.284			1.48							1.57	1.47	1.52
6	*trans*-Pinocarveol	Monoterpene	$C_{10}H_{16}O$	152.23	6.374	1.53		1.29	2.58	2.49	2.33	2.00	2.38		1.77	1.65	1.44
7	delta-Terpineol	Monoterpene	$C_{10}H_{18}O$	154.25	6.722					3.78							1.37
8	p-Mentha-1,3,8-triene	Monoterpene	$C_{10}H_{14}$	134.22	7.007		4.98	5.02	6.93	6.43	6.48	5.90	6.07	5.47	4.99		
9	*cis*-p-mentha-1(7),8-dien-2-ol	Monoterpene	$C_{10}H_{16}O$	152.23	7.007	4.74										5.28	5.36
10	*cis*-Carveol	Monoterpene	$C_{10}H_{16}O$	152.23	7.438			1.57								1.56	1.81
11	Cyclohexanol, 2-methylene-5-(1-methylethenyl)-	Monoterpene	$C_{10}H_{16}O$	152.23	7.556		3.27	3.45	3.90		3.66	3.90	3.84	3.49	3.64	4.13	
12	Carvol	Monoterpene	$C_{10}H_{14}O$	150.22	7.799												0.91
13	Cyclohexane, tert-pentyl-	Alkyl	$C_{11}H_{22}$	154.29	8.904	5.44	3.71	3.79	3.15	2.95							
14	m-Mentha-1,8-diene	Monoterpene	$C_{10}H_{16}$	136.23	9.182	5.64	4.08	3.65									
15	1-Methyl-4-isopropyl-*cis*-3-hydroxycyclohex-1-ene-6-one	Monoterpene	$C_{10}H_{16}O_2$	168.23	10.009	14.83	14.15	15.62	10.18	8.29	9.15	10.81	8.78	8.62	6.61	6.15	6.21
16	4-Propoxycatechol	Phenols	$C_9H_{12}O_3$	152.19	10.085	6.03	6.84	7.51	4.93	3.97	4.63		4.22	4.28			3.13
17	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, *trans*-	Monoterpene	$C_{10}H_{16}O_2$	168.23	10.210	1.98		2.74				5.37			2.81	2.80	1.98
18	(+) spathulenol	Sesquiterpene	$C_{15}H_{24}O$	220.35	12.135												1.35
19	Neophytadiene	Diterpenoids	$C_{20}H_{38}$	278.5	14.838										1.75	1.95	1.15
20	Hexahydrofarnesyl acetone	Ketones	$C_{18}H_{36}O$	268.5	14.908										1.47	1.74	1.47
21	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-	Diterpenoids	$C_{18}H_{30}O$	262.4	15.686										1.73	1.50	1.05
22	Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.42	16.052	1.63	2.05	1.54				2.55	2.74		4.14	3.79	3.25
23	Phytol	Diterpenoids	$C_{20}H_{40}O$	296.53	17.506				2.40	2.24	2.69	3.58	3.31	3.55	7.74	8.02	6.44
24	Linolenic acid	Fatty acid	$C_{18}H_{30}O_2$	278,4	17.743										3.01	2.68	2.48
25	Erucyl amide	Amide fatty acids	$C_{22}H_{43}NO$	337.6	22.871		4.50	3.32	3.65		5.87	3.66	5.77	6.80	3.56	3.60	2.59
26	.+/-.-2-Methoxy-3,8-dioxocephalotax-1-ene	Alkaloids	$C_{18}H_{17}NO_5$	327.3	24.476				10.90	6.63	12.76						
27	5-Hydroxy-3,7,4'-trimethoxyflavone	Flavonols: Kaempferol	$C_{18}H_{16}O_6$	328.3	24.488	11.70	13.95	12.21				16.19	16.62	18.83	13.53	12.55	10.05

MF - molecule formula; MW - molecule weight (g mol^{-1}); RT - retention time (min); T1 - (TN+0 g N fertilizer polybag^{-1}); T2 - (TN+0.9 g N fertilizer polybag^{-1}); T3 - (TN+1.36 g N fertilizer polybag^{-1}); T4 - (25% shade+0 g N fertilizer polybag^{-1}); T5 - (25% shade+0.9 g N fertilizer polybag^{-1}); T6 - (25% shade+1.36 g N fertilizer polybag^{-1}); T7 - (50% shade+0 g N fertilizer polybag^{-1}); T8 - (50% shade+0.9 g N fertilizer polybag^{-1}); T9 - (50% shade+1.36 g N fertilizer polybag^{-1}); T10 - (75% shade+0 g N fertilizer polybag^{-1}); T11 - (75% shade+0.9 g N fertilizer polybag^{-1}); T12 - (75% shade+1.36 g N fertilizer polybag^{-1})

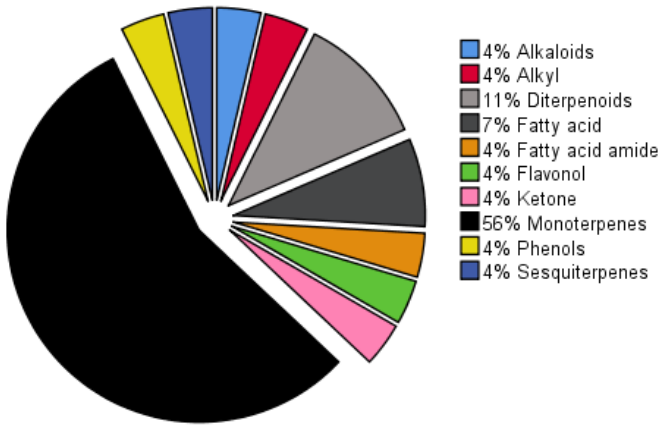


Figure 3. Percentage of compound groups found in ethanol extract of Java cardamom leaves in 12 treatment combinations

analysis showed that the 1.8-Cineole compound was the main component of the 12 treatment combinations of Java cardamom leaf extract: T1 (23.49%), T2 (22.88%), T3 (19.53%), T4 (33.35%), T5 (36.87%), T6 (35.45%), T7 (18.19%), T8 (19.38%), T9 (22.97%), T10 (15.88%), T11 (14.86%), and T12 (12.69%). Abdullah et al. (2017) reported that α-terpinyl acetate (38.4%) and 1.8-Cineole (28.71%) were also the main components in the essential oil of green cardamom (*E. cardamomum*). The various concentration values of the 1.8-Cineole compound from previous studies showed that the treatment given, the type of plant species, the location of growth, the part of the plant, and the solvent used affected the concentration of each compound in the tested plant extracts.

The highest production of the 1.8-Cineole compound was obtained in the combination of 25% shade treatment with

0.9 g N fertilizer per polybag, followed by a combination of 25% shade treatment with 1.36 g N fertilizer per polybag in the ethanol extract of Java cardamom leaves. Compound 1.8-Cineole is a natural monoterpene compound known as eucalyptol that has antioxidant and anticancer activity (Cai et al., 2020). Compound 5-Hydroxy-3,7,4'-trimethoxyflavone belongs to the kaempferol group, which was found at RT 24.488 in the ethanol extract of Java cardamom leaves treated with T1 (11.7%), T2 (13.95%), T3 (12.21%), T7 (16.19%), T8 (16.62%), T9 (18.83%), T10 (13.53%), T11 (12.55%), and T12 (10.05%). The compound 5-Hydroxy-3,7,4'-trimethoxyflavone has been reported to have antibacterial, anti-inflammatory, antimicrobial, and antioxidant activities (Sudha et al., 2016; Sudha et al., 2018; Macedo et al., 2019). The chromatogram results of the ethanol extract of Java cardamom leaves (Table 3) showed that the T4 (10.9%), T5 (6.63%), and T6 (12.76%) treatments contained the compound +/-.-2-Methoxy-3,8-dioxocephalotax-1-ene, which is an alkaloid compound. Okoh et al. (2010) also reported that compound +/-.-2-Methoxy-3,8-dioxocephalotax-1-ene has antibacterial activity.

In treatments T3, T11, and T12, cis-Carveol compounds, belonging to the monoterpene group and having antioxidant activity, were identified at an RT of 7.438 in the ethanol extract of Java cardamom leaves (1.57, 1.56, and 1.81%, respectively). The cis-Carveol compound was reported to reduce oxidative stress in hippocampal rats caused by Aβ1-42 (Hritcu et al., 2020). At RT 16.052, Hexadecanoic acids were found in treatments T1 (1.63%), T2 (2.05%), T3 (1.54%), T7 (2.55%), T8 (2.74%), T10 (4.14%), T11 (3.79%), and T12 (3.25%), which have antioxidant potential (Mazumder et al., 2020). The (+) Spathulenol compound has biological activity, such as anti-inflammatory and antimicrobial activities (Dib et al., 2017), and it was found in the ethanol extract of Java cardamom leaves at RT 12.135 in treatment T12 (1.35%). Nascimento et al. (2018) also reported that the spathulenol compound had high antioxidant activity using the DPPH and MDA methods, with an IC_{50} value of 26.13-85.60 μg mL^{-1}.

The p-Cymene and o-Cymene compounds were found with RTs of 4.811 and 4.790, respectively, in the ethanol extract of Java cardamom leaves treated with T9 (2.57%) and T12 (0.91%). In comparison, o-Cymene, which has antioxidant activity, was found after treatment with T1 (2.99%), T2 (2.96%), T3 (2.48%), T4 (4.41%), T5 (3.3%), T6 (4.37%), and T7 (2.33%). Oliveira et al. (2015) reported that p-Cymene has antioxidant potential and can act as a neuroprotective agent in the brain. This is also supported by Julaeha et al. (2020), who reported that p-Cymene and o-Cymene are responsible for the antioxidant activity of *C. limon* EO. At RT 6.284, cis-p-Menth-2,8-dienol, which has anticancer, antimicrobial, and antitumor potential (Nayak et al., 2014), was found after treatment with T1 (4.74%), T11 (5.28%), and T12 (5.36%). In addition, Ambrosio et al. (2021) also reported that cis-p-Mentha-2,8-dien-1-ol and trans-p-Menth-2,8-dienol showed the highest antibacterial and antioxidant activity. Trans-p-Menth-2,8-dienol was found in the ethanol extract of Java cardamom leaves under all treatments but in different concentrations: T1 (3.64%), T2 (3.46%), T3 (3.32%), T4 (4.35%), T5 (4.32%), T6 (4.35%), T7 (3.92%), T8 (5.1%), T9 (4.6%), T10 (3.69%), T11 (3.93%), and T12 (3.8%).

Based on secondary metabolite profile analysis and multivariate analysis, it was found that several compounds also act as antioxidants in the ethanol extract of Java cardamom leaves, namely compounds 1,8-Cineole (M3), trans-p-Mentha-2,8-dienol (M4), and 1 -Methyl-4-isopropyl-cis-3-hydroxycyclohex-1-ene-6-one (M15), which were present in each treatment combination. The chemometric analysis concluded that 1-Methyl-4-isopropyl-cis-3-hydroxycyclohex-1-ene-6-one (M15), 4-Propoxycatechol (M16), Cyclohexane, tert-pentyl- (M13), cis-p- Menth-2,8-dienol (M9), cis-Carveol (M10), and cis-p-mentha-1(7),8-dien-2-ol (M5) are the metabolites responsible for antioxidant activity in the ethanol extract of Java cardamom leaves. Interestingly, the combination of 75% shade treatment with 0 g N fertilizer per polybag (T10) had the highest FRAP antioxidant activity compared to the other treatment combinations. In comparison, the combination of 0% shade treatment with 1.36 g N fertilizer per polybag (T3) had the highest DPPH antioxidant activity compared to the other treatments.

Based on principal component analysis (PCA), two principal components were identified (Figure 4A). Principal component 1 (PC-1) explained 41.3% of the variance, and principal component 2 (PC-2) explained 17.4% of the variance, totaling 58.7%. The two PC values showed a weak two-dimensional visualization because the PC-1 and PC-2 values were less than 70% (Jolliffe & Cadima, 2016); therefore, a dendrogram hierarchical cluster analysis is needed to describe the data better (Péroumal et al., 2017). PCA analysis results showed that T12 (N3P2), T11 (N3P1), T10 (N3P0), T7 (N2P0), T8 (N2P1), T4 (N2P2), and T5 (N1P1) were dominant in PC-1, while T1 (N0P0), T3 (N0P2), and T2 (N0P1) were dominant in PC-2 (Figure 4A).

The score plot results (Figure 4A) showed three main groups out of 12 treatment combinations of ethanol extract of Java cardamom leaves based on the GC-MS phytochemical profile and antioxidant activity. The yellow circle indicates the first group, consisting of extracts of Java cardamom leaves from treatments T12 (N3P2), T11 (N3P1), and T10 (N3P0). The second group is indicated by a purple circle, consisting of an extract of Java cardamom leaves from treatments T7 (N2P0), T8 (N2P1), T9 (N2P2), T6 (N1P2), T4 (N1P0), and T5 (N1P1), while the red circle shows that the third group consisted of extracts of Java cardamom leaves from treatments T1 (N0P0), T3 (N0P2), and T2 (N0P1).

Biplot analysis of the phytochemical profile and antioxidant activity prepared from the comparison of PC-1 and PC-2 components showed three groups (Figure 4C). FRAP antioxidant activity was more dominant in group 1, consisting of ethanol extract of Java cardamom leaves from treatments T12 (N3P2), T11 (N3P1), and T10 (N3P0) with the highest metabolite content of cis-p-Menth-2,8-dienol (M5), cis-Carveol (M10), cis-p-mentha-1(7),8-dien-2-ol (M9), Hexadecanoic acid (M22), (+) spathulenol (M18), Carvol (M12), 2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methyl ethyl)-, trans- (M17), Neophytadiene (M19), Hexahydrofarnesyl acetone (M20), 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl- (M21), Linolenic acid (M24),

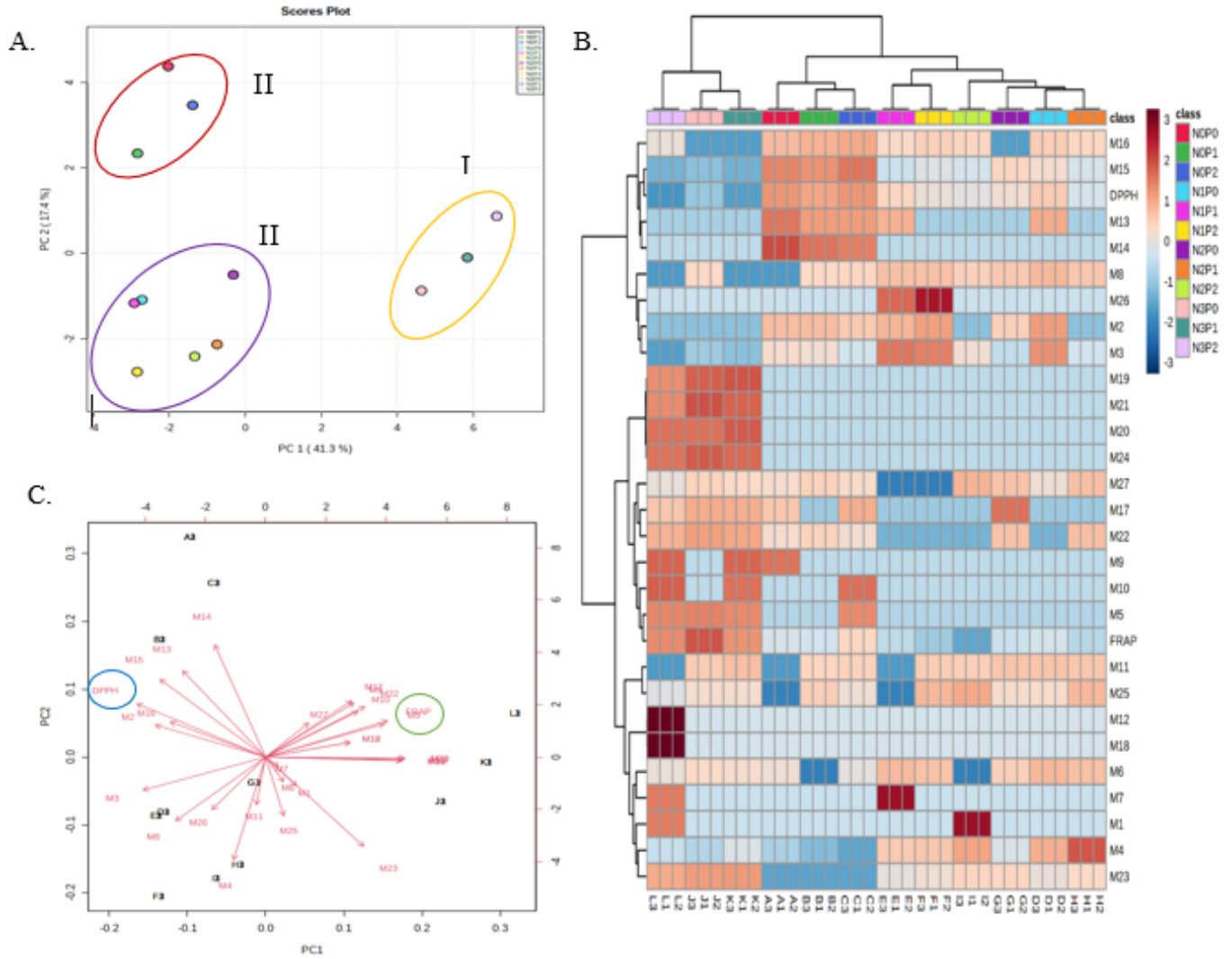


Figure 4. (A) Score plot; (B) loading plot; and (C) HCA-heatmap dendrogram of PCA on 12 treatment combinations of Java cardamom leaf extract using metabolites (M1-M47, see Table 3) and DPPH and FRAP antioxidant activities as input variables. The darker red, orange, and darker blue presented higher, moderate, and lower metabolite contents and antioxidant activities, respectively. (For an explanation of symbols, see Table 2)

and 5-Hydroxy-3,7,4'-trimethoxyflavone (M27). Group 2 consisted of ethanol extract of Java cardamom leaves from treatments T7 (N2P0), T8 (N2P1), T9 (N2P2), T6 (N1P2), T4 (N1P0), and T5 (N1P1), which had the highest metabolite content of p-Mentha-1,3,8-triene (M8), .+/-.-2-Methoxy-3,8-dioxocephalotax-1-ene (M26), trans-p-Mentha-2,8-dienol(M4), Cyclohexanol, 2-methylene-5-(1-methyl ethenyl)- (M11), Erucyl amide (M25), Phytol (M23), trans-Pinocarveol (M6), delta-Terpineol (M7), p-Cymene (M1), and 1.8-Cineole (M3). In comparison, the DPPH antioxidant activity was more dominant in group 3, which consisted of Java cardamom leaf extract from treatments T1 (N0P0), T3 (N0P2), and T2 (N0P1), with the highest metabolite content of o-Cymene (M2), 4-Propoxycatechol (M16), 1-Methyl-4-isopropyl-cis-3-hydroxycyclohex-1-ene-6-one (M15), Cyclohexane, tert-pentyl (M13), and m-Mentha-1,8-diene (M14).

Based on the dendrogram hierarchical cluster analysis, it was shown that toward a value of 3 (red color), the phytochemical content and antioxidant activity had a high correlation in the 12 ethanol extracts of Java cardamom leaves. In contrast, values near -3 (blue) showed a lower correlation. Based on the similarity of the phytochemical content and antioxidant activity, the 12 treatment combinations in the ethanol extract of Java cardamom leaves were divided into three groups (Figure 4B), namely group 1 consisted of 6 treatment combinations (N2P2, N1P0, N2P0, N2P2, N1P2, and N1P1), which contained metabolites delta-Terpineol (M7) (1.37-3.78%), p-Cymene (M1) (0.91-2.57%), .+/-.-2-Methoxy-3,8-dioxocephalotax-1-ene (M26) (6.63-12.76%), and trans-p-Mentha-2,8-dienol (M4) (3.32-5.10%) which were higher than the other treatment combinations in the ethanol extract of Java cardamom leaves. However, they had DPPH antioxidant activity and relatively weak FRAP activity compared to other treatment combinations.

Group 2 consisted of 3 treatment combinations (T3 (N0P2), T2 (N0P1), and T1 (N0P0)), which had the highest metabolite content of m-Mentha-1,8-diene (M14) (3.65-5.64%), 1-Methyl-4-isopropyl-cis- 3-hydroxycyclohex-1-ene-6-one (M15) (6.15-15.62%), Cyclohexane, tert-pentyl- (M13) (2.95-5.44%), and 4-Propoxycatechol (M16) (3.13-7.51%) and an intense DPPH antioxidant activity of 0.96-1.00 µmol TEAC

g^{-1} DW compared to other treatment combinations in the ethanol extract of Java cardamom leaves. Group 3 consisted of 3 treatment combinations, T11 (N3P1), T10 (N3P0), and T12 (N3P2), which contained metabolites Carvol (M12) (0.91%), (+) spathulenol (M18) (1.35%), Neophytadiene (M19) (1.15-1.95%), 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl- (M21) (1.05-1.73%), Hexahydrofarnesyl acetone (M20) (1.47-1.74%), Linolenic acid (M24) (2.48-3.01%), cis-p-mentha-1(7),8-dien-2-ol (M9) (4.74-5.36%), and cis-Carveol (M10) (1.57-1.81%). FRAP antioxidant activity was the strongest compared to the other groups (5.61-6.12 µmol TEAC g^{-1} DW). The diversity of phytochemical content and DPPH and FRAP antioxidant activity in the ethanol extract of Java cardamom leaves under the 12 combination treatments with different shade levels and N fertilizer doses can be used in cultivation, especially those related to solid antioxidant activity.

Compound 1.8-Cineole was the main component in the ethanol extract of Java cardamom leaves. The concentration of the 1.8-Cineole compound varied for each treatment combination; the combination of 25% shade with 0.9 g N fertilizer per polybag (T5) had the highest concentration (36.87%). This shows that shading treatment with N fertilizer can affect the content of secondary metabolites, especially 1.8-Cineole. In addition, the compound 5-Hydroxy-3,7,4'-trimethoxyflavone was also reported to have DPPH and FRAP antioxidant activity with IC_{50} values of 0.208 and 0.322 M mL^{-1}, respectively; values of 0.185 and 0.339 M mL^{-1}, respectively, were obtained from Plectranthus flandulosus Hook. (Lamiaceae) (Tsopmejio et al., 2019). Sudha et al. (2016) and Macedo et al. (2019) also reported that 5-Hydroxy-3,7,4'-trimethoxyflavone has anti-inflammatory and antibacterial activity.

Conclusions

1. Use of 75% shade had the highest TPC, TFC, and FRAP antioxidant activity in the ethanol extract of Java cardamom leaves compared to the other treatments. In contrast, the highest DPPH antioxidant activity was obtained under 0% shade.

2. There were 27 compound components in the ethanol extract of Java cardamom leaves, with compounds 1.8-Cineole (36.87%) and 5-Hydroxy-3,7,4'-trimethoxyflavone (18.83%) being the dominant metabolites. The highest content was obtained in the combination treatments of 25% shade with 0.9 g N fertilizer per polybag and 50% shade with 1.36 g N fertilizer per polybag.

3. 1-Methyl-4-isopropyl-cis-3-hydroxycyclohex-1-ene-6-one, 4-Propoxy-catechol, Cyclohexane, tert-pentyl-, cis-p-Menth -2,8-dienol, cis-Carveol, and cis-p-mentha-1(7),8-dien-2-ol were the metabolites responsible for antioxidant activity in the ethanol extract of Java cardamom leaves.

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Literature Cited

Abdullah, A.; Butt, M.S.; Shahid, M.; Asghar, A.; Shahid, M.; Huang, Q. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*. Journal of Food Science and Technology, v.54, p.2306-2315, 2017. https://doi.org/10.1007%2Fs13197-017-2668-7

Alagupalamuthirsolai, M.; Ankegowda, S. J.; Krishnamurthy, K. S. Effect of different shade levels on growth, physiology and biochemical characteristics of small cardamom (*Elettaria cardamomum* Maton). Current Journal of Applied Science and Technology, v.28, p.1-9, 2018. https://doi.org/10.9734/CJAST/2018/42040

Ambrosio, C. M. S.; Diaz-Anrenas, G. L.; Agudelo, L. P. A.; Stashenko, E.; Contreras-Castillo, C.J.; Gloria, E.M.D. Chemical composition and antibacterial and antioxidant activity of a citrus essential oil and its fractions. Molecules, v.26, p.1-16, 2021. https://doi.org/10.3390/molecules26102888

Arista, R. A.; Prioseoryanto, B. P.; Nurcholis, W. Profile volatile compounds in essential oils on different parts of cardamom with antioxidant activity. Biointerface Research in Applied Chemistry, v.13, p.1-17, 2023b. https://doi.org/10.33263/BRIAC134.328

Arista, R. A.; Prioseoryanto, B. P.; Nurcholis, W. Total phenolic, flavonoids contents, and antioxidant activities in the stems and rhizomes of Java cardamom as affected by shading and N fertilizer dosages. Yuzuncu Yil University Journal of Agricultural Sciences, v.31, p.29-39, 2023a. http://doi.org/10.29133/yyutbd.1193221

Cai, Z. M.; Peng, J. Q.; Chen, Y.; Tao, L.; Zhang, Y. Y.; Fu, L. Y.; Long, Q. D.; Shen, X. C. 1,8-Cineole: a review of source, biological activities, and application. Journal of Asian Natural Products Research, v.23, p.1-17, 2020. https://doi.org/10.1080/10286020.2020.1839432

Danet, A. F. Antioxidant-benefits, sources, mechanisms of action. IntechOpen, v.1, p.1-646, 2021. https://doi.org/10.5772/intechopen.92918

Dib, I.; Fauconnier, M. L.; Sindic, M.; Belmekki, F.; Assaidi, A.; Berrabah, M.; Mekhfi, H.; Aziz, M.; Legssyer, A.; Bnouham, M.; Ziyyat, A. Chemical composition, va-sorelaxant, antioxidant and antiplatelet effects of essential oil of *Artemisia campestris* L. from Oriental Morocco. BMC Complement and Alternative Medicine, v.17, p.1-15, 2017.

Hritcu, L.; Boiangiu, R. S.; Morais, M. C. D.; Sousa, D. P. D. (-)-*cis*-Carveol, a natural compound, improves β-Amyloid-Peptide 1-42-induced memory impairment and oxidative stress in the rat hippocampus. BioMed Research International, p.1-9, 2020. https://doi.org/10.1155/2020/80825

Jolliffe, I. T.; Cadima, J. Principal component analysis: a review and recent developments. Philosophical Transactions of the Royal Society A, v.374, p.1-16, 2016.

Julaeha, E.; Dewi, K. S.; Nurzaman, M.; Wahyudi, T.; Herlina, T.; Hardianto, A. Chemical compositions and antioxidant activities of Indonesian citrus essential oils and their elucidation using principal component analysis. Preprints, p.1-11, 2020.

Leghari, S. J.; Wahocho, N. A.; Laghari, G. M.; Hafeez-Laghari, A.; Mustafa, Bhabhan, G.; Hussain-Talpur, K.; Bhutto, T. A.; Wahocho, S. A.; Lashari, A. A. Role of nitrogen for plant growth and development: a review. AENSI Journal, v.10, p.209-218, 2016.

Macedo, I.; Silva, J. Hd.; Silva, P. Td.; Cruz, B. G.; Vale, J. P. Cd.; Santos, H. Sd.; Bandeira, P. N.; Souza, E. Bd.; Xavier, M. R.; Countinho, H. D. M.; Braz-Filho, R.; Teixeira, A. M. R. Structural and microbiological characterization of 5-Hydroxy-3,7,4¢-Trimethoxyflavone: a flavonoid isolated from *Vitex gardneriana* Schauer leaves. Microbial Drug Resistance, v.25, p.1-6, 2019.

Mazumder, K.; Nabila, A.; Aktar, A.; Farahnaky, A. Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of *Australian lupin* species: a comprehensive substantiation. Antioxidants, v.9, p.1-23, 2020.

Muflihah, Y. M.; Gollavelli, G.; Ling, Y. C. Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. Antioxidants MDPI, v.10, p.1-15, 2021. https://doi.org/10.3390/antiox10101530

Nascimento, D. K. F.; Moreira, F. M. F.; Alencar, Santos, J.; Kassuya, C. A. L.; Croda, J. H. R.; Cardoso, C. A. L.; et al. Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw. and spathulenol. Journal Ethnopharmacol, v.210, p.351-358, 2018. https://doi.org/10.1016/j.jep.2017.08.030

Nayak, S.; Jena, A. K.; Mittal, D. K.; Joshi, D. GC-MS analysis of phytoconstituents of some wild *Zingiberaceae* plants methanolic rhizome extracts. Research in Plant Sciences, v.2, p.1-5, 2014.

Nurcholis, W.; Alfadzrin, R.; Izzati, N.; Arianti, R.; Vinnai, B. Á.; Sabri, F.; Kristóf, E.; Artika, I. M. Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of Java cardamom (*Amomum compactum* Soland Ex Maton) Fruit. Plants, v.11, p.1-13, 2022. https://doi.org/10.3390/plants11172221

Nurcholis, W.; Khumaida, N.; Bintang, M.; Syukur, M. GC-MS analysis of rhizome ethanol extracts from *Curcuma aeruginosa* accessions and their efficiency activities as anticancer agent. Biodiversitas, v.22, p.1179-1186, 2021b. https://doi.org/10.13057/biodiv/d220313

Nurcholis, W.; Ma'rifah, K.; Artika, M. I.; Aisyah, S. I.; Priosoeryanto, B. P. Optimization of total flavonoid content from cardamom fruits using a simplex-centroid design, along with the evaluation of the antioxidant properties. Tropical Journal of Natural Product Research, v.5, p.1382-1388, 2021c. http://doi.org/10.26538/tjnpr/v5i8.10

Nurcholis, W.; Putri, D. S.; Husnawati, H.; Aisyah, S. I.; Priosoeryanto, B. P. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. Annals of Agricultural Sciences, v.66, p.58-62, 2021a. https://doi.org/10.1016/j.aoas.2021.04.001

Okoh, O. O.; Sadimenko, A. P.; Afolayan, A. J. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chemistry, v.120, p.308-312, 2010. http://doi.org/10.1016/j.foodchem.2009.09.084

Oliveira, T. M. D.; Carvalho, R. B. F. D.; Costa, I. H. F. D.; Oliveira, G. A. L. D.; Souza, A. A. D.; Lima, S. G. D. L.; Freitas, R. M. D. Evaluation of p-cymene, a natural antioxidant. Pharmaceutical Biology, v.53, p.423-428, 2015. https://doi.org/10.3109/13880209.2014.923003

Péroumal, A.; Adenet, S.; Rochefort, K.; Fahrasmane, L.; Aurore, G. Variability of traits and bioactive compounds in the fruit and pulp of six mamey apple (*Mammea americana* L.) accessions. Food Chemistry, v.234, p.269-275, 2017. http://doi.org/10.1016/j.foodchem.2017.04.145

Sivakumar, D.; Jifon, J. Influence of photoselective shade nettings on postharvest quality of vegetables. In: Preharvest modulation of postharvest fruits and vegetable quality. Boca Raton, FL, USA: AAP-CRC Press; Amsterdam, The Netherlands: Elsevier Inc., 2017. p.121-138.

Sudha, A.; Jeyakanthan, J.; Srinivasan, P. Protective effect of 5-hydroxy-3',4',7-trimethoxyflavone against inflammation induced by lipopolysaccharide in RAW 264.7 macrophage: *in vitro* study and *in silico* validation. Medicinal Chemistry Research, v.25, p.1754-1767, 2016.

Sudha, A.; Srinivasan, P.; Kanimozhi, V.; Palanivel, K.; Kadal-mani, B. 2018. Antiproliferative and apoptosis-induction studies of 5-hydroxy 3',4',7-trimethoxyflavone in human breast cancer cells MCF-7: an *in vitro* and *in silico* approach. Journal of Receptors and Signal Transduction Research, v.38, p.179-190, 2018. https://doi.org/10.1080/10799893.2018.1468780

Suhendra, C. P.; Widarta, I. W. R.; Wiadnyani, A. A. I. S. The effect of ethanol concentration on antioxidant activity of cogon grass rhizome (*Imperata cylindrica* (Linn.) Beuv.) extract using ultrasonic wave. Jurnal Ilmu dan Teknologi Pangan, v.8, p.27-35, 2019.

Tarfaoui, K.; Brhadda, N.; Ziri, R.; Oubihi, A.; Imtara, H.; Haida, S.; Al kamaly, O.M.; Saleh, A.; Parvez, M. K.; Fettach, S.; et al. Chemical profile, antibacterial and antioxidant potential of *Zingiber officinale* Roscoe and *Elettaria cardamomum* (L.) Maton essential oils and extracts. Plants, v.11, p.1-14, 2022. https://doi.org/10.3390/plants11111487

Tsopmejio, J. P.; Momeni, J.; Tsopjio, F. N.; Tedjon, V. S.; Mkounga, P.; Shehla, N.; Choudhary, M. I.; Nkengfack, A. E. Bioactive secondary metabolites from *Plectranthus glandulosus* Hook. (Lamiaceae). Phytochemistry Letters, v.30, p.133-137, 2019. https://doi.org/10.1016/j.phytol.2019.01.029