Indonesian Journal of Agronomy

ISSN 2085-2916 | e-ISSN 2337-3652
Indonesian J. Agron., *51*(2), 221-233, August 2023
DOI: https://dx.doi.org/10.24831/ija.v51i2.44781

Research Article

Accession variation in starch, chlorophyll, and antioxidant content of cardamom (*Amomum cardamomum*) leaves

Lelly Aulia Damarhati[1], **Stephanie Sriwongo**[1], **Aulia Taureza**[1], **Teti Mulyati**[1], **Novian Liwanda**[1], **and Waras Nurcholis**[1,2,*]

[1] Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Agatis, Kampus IPB Dramaga, Bogor, 16680, West Java, INDONESIA
[2] Tropical Biopharmaca Research Center, IPB University, Jl. Taman Kencana, Bogor, 16128, West Java, INDONESIA
* Corresponding author (✉ wnurcholis@apps.ipb.ac.id)

ABSTRACT

Cardamom (Amomum cardamomum) is a herbal plant with various phytochemical compounds such as phenols, starch, tannins, terpenoids, flavonoids, and antioxidants. Cardamom of the same type but originating from different regions can produce variations in the content of metabolites. This study aimed to select cardamom accession containing high starch, chlorophyll, and antioxidant capacity. The research was conducted at Biopharmaca Experimental Garden, Cikabayan IPB University from March to August 2022 using four cardamom accessions, i.e., Bogor Hijau, Bogor Merah, Ciamis and Sukabumi. Three leaves were taken for each accession, with three replications. The leaves taken were numbers 5, 6, and 7 counted from the tops of the cardamom plant. The methods used in this study were the phenol-sulfuric acid method, the DMSO method, the DPPH method, and the CUPRAC method. The results showed that the highest starch content was produced by the cardamom of Bogor Merah accession, with an average of 3.63 g (100 g)$^{-1}$ FW. The highest total chlorophyll content was found in the cardamom of Bogor Hijau accession, with an average of 1.9 mg g^{-1} FW. At the same time, the highest antioxidant capacity using the DPPH method was produced by the cardamom of the Sukabumi accession with an average of 1.28 µmol TE g^{-1} FW. In comparison, the highest antioxidant capacity of the CUPRAC method was produced by the cardamom of the Bogor Hijau accession with an average of 5.71 µmol TE g^{-1} FW. Thus, it is recommended to use Bogor Merah dan Bogor Hijau accession for further evaluation.

Keywords: accession, antioxidant activity, cardamom leaves, chlorophyll, starch

Edited by:
Maryati Sari

Received:
11 December 2022
Accepted:
3 August 2023
Published online:
21 August 2023

Citation:
Damarhati, L. A., Sriwongo, S., Taureza, A., Mulyati, T., Liwanda, N., & Nurcholis, W. (2023). Accession variation in starch, chlorophyll, and antioxidant content of cardamom (Amomum cardamomum) leaves . *Indonesian Journal of Agronomy, 51*(2), 221-233

INTRODUCTION

Cardamom (*Amomum cardamomum*) is a type of herbal plant from a group of shrubs from the Zingiberaceae family. The cardamom plant has distinctive characteristics such as pseudo stems, round fruit and green tillers. Cardamom plants generally have a height of about 1.5 m with a characteristic single leaf on each stem. The green leaves attached to cardamom have a pinnate shape on the bones, with a length of about 25-35 cm and a width of about 10-12 cm (Alqamari et al., 2017). This plant is known to have properties as traditional medicine and has the potential as an export commodity. Cardamom also has economic value in other fields, such as cooking and cosmetics (Nurzaman et al., 2020).

Cardamom is one of the essential spice commodities in Southeast Asia because of its sizeable contribution to the total production of biopharmaceutical plants in Indonesia. The total production of cardamom in Indonesia in 2020 was 62,923 ton with the export volume reaching 6,248 tons or US$ 8 million (Suhartini et al., 2021). Indonesia itself has two types of cardamom that are best known in society, namely cardamom across (*Elettaria cardamomum*) and Javanese cardamom (*Amomum compactum*) (Setyawan et al., 2014). These two types of cardamom are differentiated based on agroecology. Javanese cardamom is native to Indonesia and becomes primary cardamom production because more adaptive and easy to cultivate. Cardamom across is less tolerant to dry environments (Nurzaman et al., 2020), thus more difficult to cultivate in Indonesia.

According to Borges et al. (2017), the same plant species can contain various metabolites. Variations in the content of these metabolites are related to the factors that influence them, such as the environmental conditions in which plants grow (Komala et al., 2022). The synthesis of phytochemical compounds in plants can be induced by interactions between plants and the environment in which they grow. The growing environment of these plants can be affected by the presence of other organisms, ambient temperature, rainfall, humidity, solar radiation, and the number of nutrients in the soil. All of the previously mentioned can affect the resulting plant metabolites (Putri, 2020). In addition, a growing environment with high levels of carbon dioxide (CO_2) can increase the production of metabolites in plants, especially plant secondary metabolites, as a form of self-protection from environmental stress (Utomo et al., 2020). In cardamom, both environmental and genetic factors affect metabolites profile (Setyawan et al., 2014).

Cardamom is one of the most popular spice plants in Indonesia. The selection of quality cardamom accessions is essential to get good production results. In selecting quality cardamom accessions, chlorophyll, starch, and antioxidant capacity levels should be the primary considerations. The accession of cardamom, which has high levels of chlorophyll and starch and good antioxidant capacity, can improve product quality and provide health benefits for consumers. Chlorophyll is necessary for photosynthesis, the process by which plants convert solar energy into food (Tanaka & Tanaka, 2011). Chlorophyll also helps provide glucose due to photosynthesis, which can be used as an ingredient in starch formation and antioxidant compounds such as phenolics and flavonoids (Jha, 2019). Research on cardamom plants from various regions of origin (accessions) needs to be conducted to facilitate the cultivation of cardamom plants. This study aimed to select cardamom accession containing high starch, chlorophyll, and antioxidant capacity.

MATERIALS AND METHODS

Cardamom planting was carried out at Biopharmaca Experimental Garden, Cikabayan IPB from March to August 2022. Evaluation of starch, chlorophyll and antioxidant levels were tested at the IPB Agricultural Biochemistry Laboratory from August to October 2022.

The monthly climate in Bogor during research was presented in Table 1. Rainfall is the amount of rainwater that falls in a particular area in a specific unit or period (daily, weekly, monthly, or yearly) as measured in millimeters (mm) (Ruhiyat, 2022). Air temperature is often assumed to vary with the free atmosphere temperature, and the time interval rate is considered typical of the free atmosphere (Heynen et al., 2016). Humidity is defined as the percentage ratio between the partial water vapor pressure and saturated water vapor pressure (Indarwati et al., 2019).

Sampling method and extraction

Four cardamom genotypes were obtained from the Bogor area (green and red cardamom types), Ciamis, and Sukabumi, corresponding to Bogor Hijau, Bogor Merah, Ciamis and Sukabumi accessions. Each accession used 30 plants planted in the Biopharmaca Experimental Garden, IPB University (-6.547030,106.715883). Plants are divided into five clumps, each clump had six plants. The spacing between plants was 10 cm, while the distance between plant clumps was one meter. Analysis was carried out after plants establish for 6 months in the field.

Table 1. Monthly data of climate in the Bogor region (BPS, 2023a; BPS, 2023b; BPS 2023c).

Month	Rainfall (mm)	Air temperature (°C)	Humidity (%)
March	113.2	26.10	85.00
April	316.6	26.40	85.00
May	228.5	26.50	85.00
June	463.7	25.50	86.00
July	358.1	26.20	80.00
August	384.9	26.10	83.00

Three leaves were taken for each accession, with three replications. The collected leaves were number 5, 6, and 7 counted from the growing points for evaluation of chlorophyll, starch and antioxidant contents. A sampling of cardamom leaves was carried out by cutting cardamom leaves using garden shears. The fresh leaves were then stored in a cooling box filled with ice for further analysis.

The leaves were ground using a fine mortar into a paste. A fresh paste of cardamom leaves, as much as 4 g, was added to 20 mL of 70% ethanol in Erlenmeyer. This mixture was heated in a microwave oven for 3 minutes at 45W power. After that, the mixture was filtered using filter paper (Whatman No. 42). The final volume of the filtered filtrate was added to 20 mL to obtain an extract concentration of 0.2 g mL^{-1}. The extract filtrate obtained will be used in the antioxidant analysis.

Analysis of starch content

As much as 0.5 g of fresh cardamom leaf paste was added to 10 mL of distilled water and stirred for 5 minutes. Then 13 mL of 52% perchloric acid ($HClO_4$) was added to the mixture and stirred again for 20 minutes using a magnetic stirrer. A total of 100 mL of distilled water was added to the mixture, then filtered and added distilled water again until the final volume was 250 mL. A total of 1 mL of sample filtrate was added with 1 mL of 5% phenol in a test tube. 5 mL of concentrated sulfuric acid (H_2SO_4) was added to this solution and immersed in water for 10 minutes. The absorbance of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 490 nm. The standard used was glucose with concentration series of 0, 15, 30, 45, 60, 80, 90, 105, and 120 ppm.

Analysis of chlorophyll levels

As much as 0.1 g of fresh cardamom leaves were cut into small pieces, then 7 mL of DMSO was added in a test tube. The mixture was heated in a water bath at 65 °C for 25 minutes. The heated filtrate was transferred to a new test tube with the addition of DMSO until the final volume was 10 mL. The blank used a solution of DMSO. Sample absorbance measurement was measured at a wavelength of 663 and 645 nm. The chlorophyll content was determined by Arnon's equation (1949), namely:

$$\text{Chlorophyll a (mg g-1)} = ([(12.7\times A663) - (2.69\times A645)] \times V)/((1000\times W))$$
$$\text{Chlorophyll b (mg g}^{-1}\text{)} = ([(22.9\times A645) - (4.68\times A663)] \times V)/((1000\times W))$$
$$\text{Total Chlorophyll (mg g}^{-1}\text{)} = ([(20.2\times A645) + (8.02\times A663)] \times V)/((1000\times W))$$

Information:
A = measured absorbance
V = volume of solution (mL)
W = fresh sample weight (g)

DPPH antioxidant test

A 100 µL of ethanol extract from fresh cardamom leaves was added to 100 µL of DPPH solution and incubated at room temperature for 20 minutes in the dark. The absorbance of the solution was measured at a wavelength of 515 nm using a nano spectrophotometer (BMG Labtech). Free radical inhibition activity was expressed in µmol TE g^{-1} fresh weight (FW) and the Trolox standard used has a range of 0-100 µmol.

Cupric Ion reducing antioxidant capacity (CUPRAC) test

A 50 µL of cardamom leaf ethanol extract was added with 50 µL $CuCl_2$ 0.01 M, 50 µL neocuproine 0.0075 M, and 50 µL ammonium acetate buffer pH 7. The solution was then incubated at room temperature for 30 minutes in the dark. The absorbance of the solution was measured at a wavelength of 450 nm using a nano spectrophotometer (BMG Labtech). The antioxidant capacity was expressed in µmol TE g^{-1} FW and the Trolox standard used has a range of 0-800 µmol.

Statistical analysis

Data on starch content, chlorophyll, and antioxidant capacity of cardamom leaves of various accessions were collected and processed using Microsoft Office Excel 2019. Data were analyzed using the ANOVA method and Tukey's post-hoc test using IBM SPSS Statistics 25. The resulting data were presented as bar charts using GraphPad Prism 8. Similarities in metabolite content between accessions were analyzed using hierarchical cluster analysis using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

Starch content

Starch is a phytochemical compound in cardamom, apart from phenols, tannins, terpenoids, flavonoids, proteins, and sterols (Moulai-Hacene et al., 2020). Starch in plant leaves functions in the repair and maintenance of stomata (Putri & Zubaidah, 2017). In addition, starch is a type of carbohydrate composed of two main components: amylose and amylopectin. Both of these polymers are composed of glucose monomers bonded to each other. This study measured the starch content of cardamom leaves using the phenol-sulfuric acid method, and glucose was used as a standard. The standard glucose curve obtained had an R^2 value of 0.9901 (Figure 1).

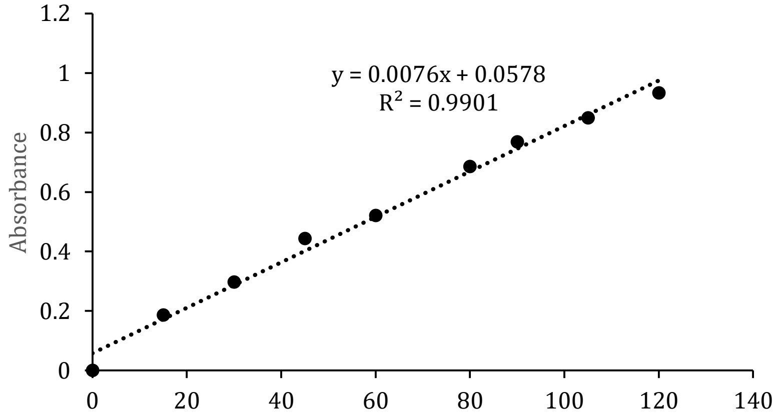


Figure 1. Correlation of glucose concentration and absorbance value in spectrophotometer.

Starch content was calculated based on the number of hydrolyzed glucose monomers from amylose and amylopectin. Starch hydrolysis is carried out with 52% perchloric acid so that the simple sugars contained in starch can react with phenol and sulfuric acid. The hydrolysis reaction between sulfuric acid and glucose will form hydroxymethyl furfural compounds and produce a yellowish-orange color when reacted with phenol (Qalsum et al., 2015). The starch content of cardamom leaves produced from various accessions is presented in Figure 2.

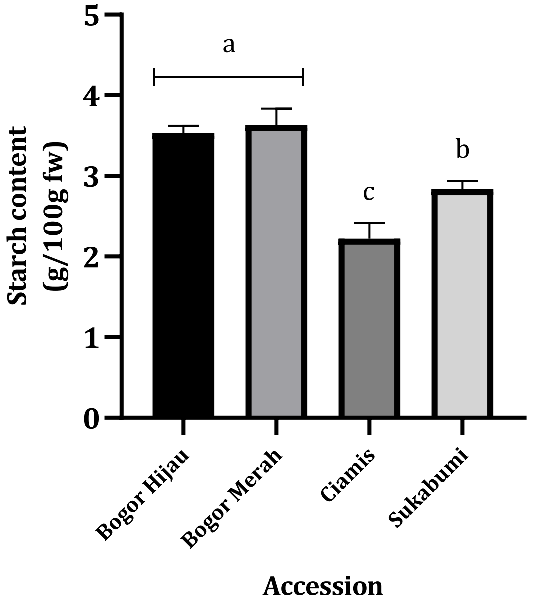


Figure 2. Starch content of cardamom accessions. Values followed by different letters are significantly different at $p < 0.05$ and Tukey test 5%. Mean ± SD.

Based on Figure 2 the starch content of the four cardamom leaf accessions, Bogor Merah was the accession with the highest starch content, with an average value of 3.63 g $(100\ g)^{-1}$ FW; followed by Bogor Hijau with an average starch content of 3.54 g $(100\ g)^{-1}$ FW and Sukabumi with an average starch content of 2.84 g $(100\ g)^{-1}$ FW. Ciamis cardamom was the accession that produced the lowest starch content of all accessions, with an average value of 2.22 g $(100\ g)^{-1}$ FW. The accessions of Bogor Merah and Bogor Hijau are similar in Tukey's test. On the other hand, Ciamis and Sukabumi accessions showed significant differences, as shown by the values that were quite far between the two. These results indicate the influence of the region of origin (accession) on the starch content produced.

The starch content in cardamom leaves is still shallow compared to starch produced by tubers and rice, such as arrowroot tubers which contain more than 85% starch (Irawan, 2012). The proportion of amylose and amylopectin undoubtedly influences the starch content in a plant. The difference in these proportions is influenced by several factors present in the location where a plant grows, such as water availability, temperature differences, soil moisture, soil type, soil pH, light intensity, harvesting age, climate, and the plant variety itself (Polnaya et al., 2015; Vinolina, 2014).

Chlorophyll content of cardamom leaves

Chlorophyll has a vital role in the formation of materials needed by plants to grow and develop, is located in the thylakoid membrane, the primary light-absorbing pigment and significantly influences plant photosynthesis (Nugroho et al., 2021). The chlorophyll content is influenced by various factors, such as plant age, genetics, and leaf morphology (Dharmadewi, 2020). In comparison, chlorophyll b is a photosynthetic antenna that collects light to be transferred to the reaction center. The light energy is quickly converted into chemical energy in the photosynthesis reaction center (Dharmadewi, 2020). The chlorophyll content analyzed in this study included chlorophyll a, b, and all chlorophyll contained in cardamom leaves. The chlorophyll content obtained from the research is presented in Figure 3.

Chlorophyll a (Figure 3a) of cardamom leaves from the four accessions showed a value range of 0.7-2.6 mg g^{-1} FW. The highest chlorophyll a was produced by Bogor Hijau cardamom (2.6 mg g^{-1} FW), while the lowest content was produced by Ciamis cardamom (0.7 mg g^{-1} FW). Furthermore, the chlorophyll b content (Figure 3b) of cardamom leaves from the four accessions showed a value range of 0.25-0.6 mg g^{-1} FW. Like the chlorophyll content, the cardamom leaves of Bogor Hijau accession also had the highest chlorophyll b content (0.6 mg g^{-1} FW), and cardamom accession Ciamis produced the lowest chlorophyll b content (0.25 mg g^{-1} FW). The total chlorophyll content (Figure 3c) of cardamom leaves from the four accessions yielded values of 0.9-1.9 mg g^{-1} FW. In this case, the highest total chlorophyll content was still produced by Bogor Hijau (1.9 mg g^{-1} FW), and the lowest total chlorophyll content was also produced by cardamom accession Ciamis (0.9 mg g^{-1} FW).

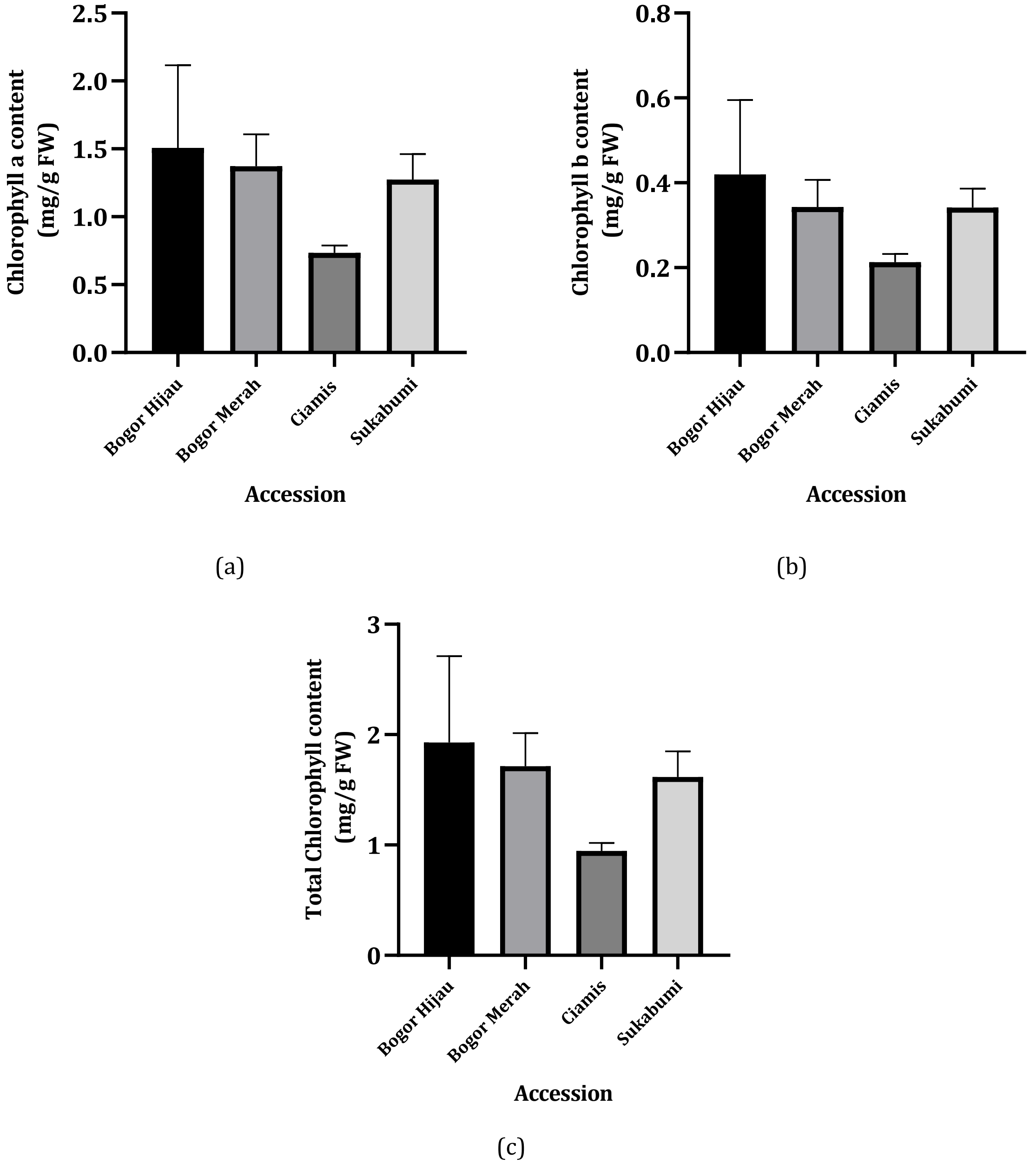


Figure 3. Chlorophyll content of cardamom of different accessions. (a) Chlorophyll a, (b) chlorophyll b, and (c) total chlorophyll content. Mean ± SD.

However, all cardamom leaf accessions showed no significant differences, either in chlorophyll a and b or in total chlorophyll. The variance supports this result in the α value, which is greater than 0.05 in the Tukey test. According to Posumah (2017), if the results of the variance analysis show that the value is more significant than 0.05, then the treatment is not significantly different from the variables analyzed. These results indicate that the influence of cardamom's region of origin (accession) does not significantly affect the resulting chlorophyll content.

DPPH antioxidant capacity

Antioxidants are compounds that play a role in inhibiting free radicals of oxygen species with unpaired electrons with reactive properties and can cause cell oxidation (Pramesti, 2013; Andarina & Djauhari, 2017). Inhibition of free radicals by antioxidants is done by binding to free radicals so that oxidation reactions do not occur. Antioxidants, including the leaves, are also found in cardamom plants (Muna et al., 2019). Antioxidant testing in this study used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and Trolox was used as a standard. The Trolox-DPPH standard curve obtained has an R^2 value of 0.921 (Figure 4). This method is based on reducing DPPH compounds with the sample's antioxidants. DPPH is a stable purple solution that can absorb light at a wavelength of 515 nm. A change in color from purple to yellow, accompanied by a decrease in absorbance at a wavelength of 515 nm, indicates that the sample can be a source of antioxidants (Mishra et al., 2012).

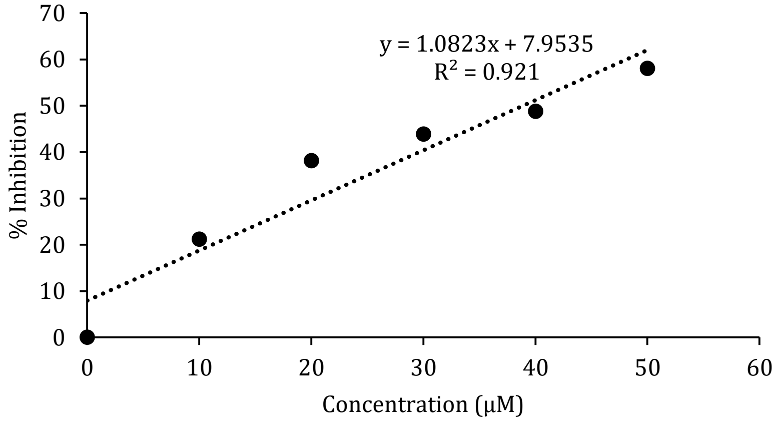


Figure 4. Correlation of Trolox-DPPH concentration and absorbance value in spectrophotometer.

Based on the results of measuring the antioxidant activity of the DPPH method (Figure 5), which was carried out on accession cardamom leaf extract, it was found that the highest antioxidant activity was cardamom Sukabumi accession, with an average value of 1.28 µmol TE g^{-1} FW. Then followed by Ciamis cardamom, which produced an average antioxidant content value of 0.81 µmol TE g^{-1} FW and Bogor Hijau cardamom, with an average antioxidant content value of 0.73 µmol TE g^{-1} FW. The lowest antioxidant activity content was found in Bogor Merah cardamom, which has an average value of 0.31 µmol TE g^{-1} FW. The results showed there was a significant difference in Tukey's test.

Meanwhile, the other two accessions formed a separate group. These results indicate that the influence of the cardamom plant's region of origin (accession) also influences the antioxidant capacity produced. However, the antioxidant capacity produced is still far below the antioxidant capacity of cardamom leaf essential oil studied by Arista et al. (2022), which is 19.07 mol TEAC g^{-1} FW. The sample type might influence this significant difference in the results analyzed because Arista et al. (2022) used essential oils extracted directly from cardamom leaves. While the antioxidant test in this study only used fresh cardamom leaf samples without essential oil extraction.

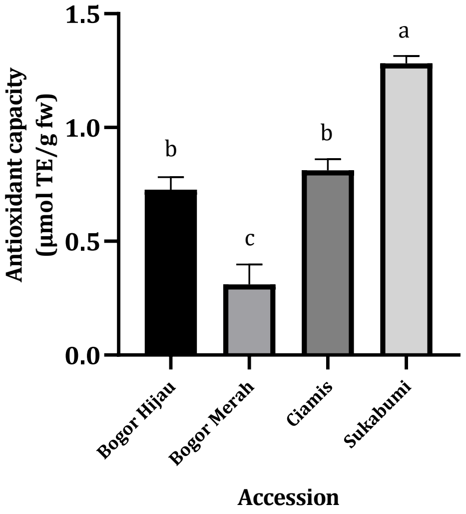


Figure 5. Antioxidant activity test using the DPPH method. Values followed by different letters are significantly different at $p < 0.05$ and Tukey test 5%. Mean ± SD.

CUPRAC antioxidant capacity

Tests for antioxidant capacity that can be done besides the DPPH method are Cupric Reducing Antioxidant Capacity (CUPRAC). The antioxidants tested by the CUPRAC method were seen based on the antioxidant power of polyphenolic compounds and vitamin E. The choice of this method for determining antioxidant capacity was based on its stability against free radicals and tended to have a low redox potential, so it is more selective (Roni et al., 2022). The principle of the CUPRAC method is electron donation with bis(neocuproine) copper (II) chelate reagent (Karahalil and Sahin, 2011). Testing with the CUPRAC method is very efficient for glutathione and thiol antioxidant types and is more selective for compounds that are not oxidized in the DPPH method. This efficiency is due to the small redox potential of Cu^{2+} metal. The smaller the redox potential, the faster the reaction (Apak et al., 2005). Measurement of the antioxidant capacity of the CUPRAC method also uses trolox as a standard with an R^2 value of 0.9893 (Figure 6).

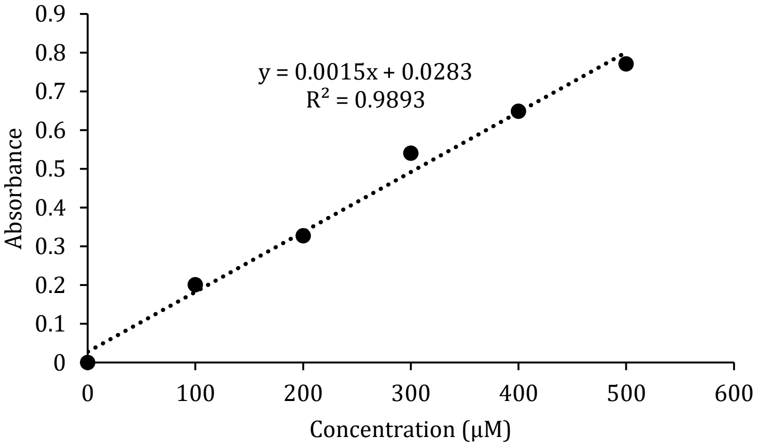


Figure 6. Correlation of Trolox- CUPRAC concentration and absorbance value in spectrophotometer.

The antioxidant capacity (reducing power) in the CUPRAC method is influenced by the flavonoid compounds contained in the sample. These flavonoid compounds play a role in reducing metal ions, for example, copper. The more vital reduction ability of an antioxidant compound indicates its higher capacity. However, the antioxidant capacity of a sample is not always directly proportional to the total flavonoid content; of course, other compounds can also act as antioxidants besides flavonoids. Other compounds contained in cardamom that can act as antioxidants include triterpenoids, saponins and tannins. This compound will reduce metal ions during the CUPRAC test (Fithriani et al., 2015).

The analysis of antioxidant capacity using the CUPRAC method showed that the highest antioxidant capacity was found in Bogor Hijau cardamom, with an average value of 5.71 µmol TE g^{-1} FW, then followed by Bogor Merah cardamom, which produced an average antioxidant capacity of 4.9 µmol TE g^{-1} FW and Sukabumi cardamom, with an average value of 2.81 µmol TE g^{-1} FW (Figure 7). The lowest antioxidant capacity was found in the Ciamis sample with an average of 2.52 µmol TE g^{-1} FW. Tukey's other test results on the antioxidant capacity of cardamom leaves based on accession showed significant differences in the four samples. This statement is proven by forming two accession groups with similar antioxidant capacities. Bogor Merah and Bogor Hijau cardamoms form one group with similar antioxidant capacities, while Ciamis and Sukabumi cardamoms form the same group with lower antioxidant capacities (Figure 8). Figure 8 shows two clusters formed with Bogor Merah and Bogor Hijau as the first cluster, and Ciamis and Sukabumi as the second cluster. These results indicate an effect of cardamom plants' accession or region of origin on antioxidant capacity.

Correlation between the antioxidant capacity with starch content and total chlorophyll

Pearson correlation analysis evaluated the degree of association between metabolites (starch and chlorophyll) and the resulting antioxidant capacity. A high degree of association (R = 0.8684) and significant ($p < 0.05$) resulted from the correlation between starch content and the antioxidant capacity of the CUPRAC method (Figure 9a). On the other hand, the degree of relationship generated by the correlation between total chlorophyll and the antioxidant capacity of the CUPRAC method yielded R = 0.5931 and a p-value of 0.04209 (Figure 9b). These results indicate that the resulting correlation between chlorophyll and CUPRAC is still lower than that of starch and CUPRAC. Meanwhile, the antioxidant capacity of the DPPH method did not have a close relationship with the two metabolites analyzed ($p > 0.05$). These results indicate the presence of other compounds responsible for the antioxidant capacity of DPPH, which were not analyzed in this study. Antioxidants in plants can be produced from compounds of the flavonoid group and other types of compounds (group of vitamins), such as ascorbic acid and α-tocopherol (Banjarnahor & Artanti, 2014; Santos-Sánchez et al., 2019).

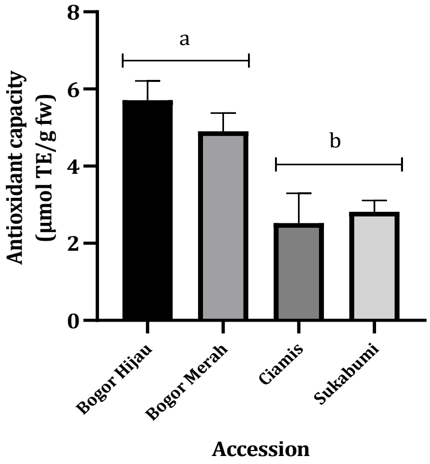


Figure 7. The results of the antioxidant activity test with the CUPRAC method. Values followed by different letters are significantly different at $p < 0.05$ and Tukey test 5%. Mean ± SD.

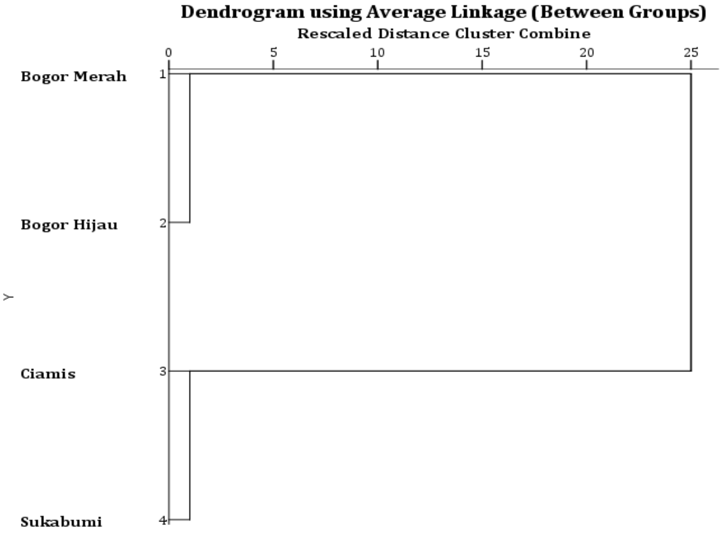
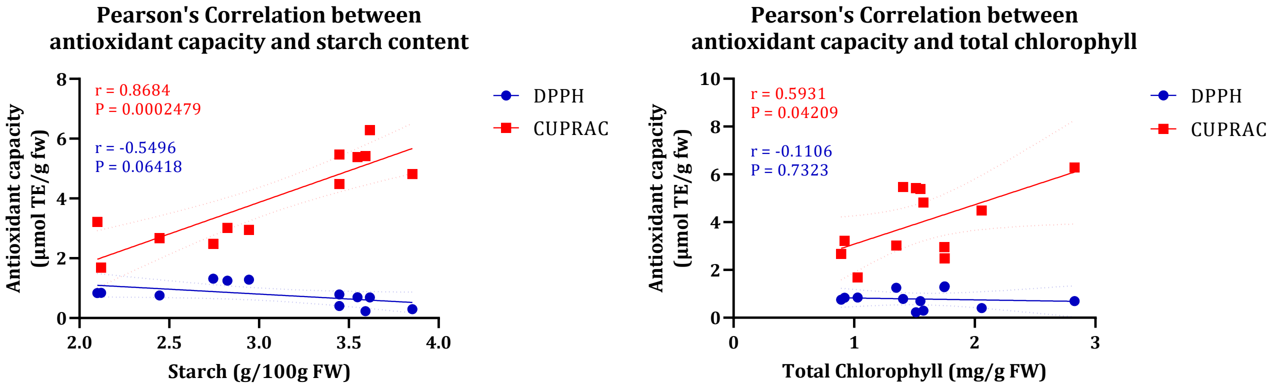


Figure 8. Results of cluster analysis of the antioxidant capacity (DPPH and CUPRAC methods) of four cardamom accessions.

The high chlorophyll content in the leaves reflects the high starch content and antioxidant capacity because the availability of glucose from photosynthesis can be fulfilled (Zhong et al., 2016). The adequacy of glucose in plants can be used as a storage material, such as starch, and to form antioxidant compounds, such as phenolics and flavonoids. Plants rich in antioxidant compounds will undoubtedly produce a high antioxidant capacity (Kschonsek et al., 2018).

The chlorophyll, starch, and antioxidant capacity content can be affected by climate change in an area (Parmesan & Hanley, 2015). Adequate rainfall will help increase chlorophyll productivity because the intensity of sunlight and the water needs needed in the photosynthesis process can be fulfilled (Du et al., 2017). In addition, suitable air temperature and humidity can promote plant growth and produce plant metabolites. Similar plants grown in different climates will certainly produce differences in metabolite content (Hatfield & Prueger, 2015). Therefore, in this study, the four cardamom accessions were planted in the same place so that no climate differences occurred in the four cardamom accessions, which could affect their metabolite content.



(a) (b)

Figure 9. Correlation analysis between metabolites and antioxidant capacity. (a) Pearson's correlation between antioxidant capacity and starch content. (b) Pearson's correlation between antioxidant capacity and total chlorophyll.

CONCLUSIONS

The metabolites content varied in cardamom accession. The cardamom accession of Bogor Merah produced the highest starch content while Bogor Hijau cardamom produced the highest chlorophyll content and antioxidant capacity in the CUPRAC method. Cardamom accession from Sukabumi produced the highest antioxidant capacity in the DPPH method. In contrast to the cardamom accession of Ciamis, which produced starch, chlorophyll and antioxidant capacity by the CUPRAC method with the lowest values among the four cardamom accessions analyzed.

ACKNOWLEDGEMENTS

The author would like to thank the Department of Biochemistry, Faculty of Mathematics and Natural Sciences IPB University for their support during the research.

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