Variability total phenolic content and antioxidant activity of *Curcuma zanthorrhiza* and *C. aeruginosa* cultivated in three different locations in West Java, Indonesia

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Abstract. Suryani, Al Anshory AC, Marlin, Artika IM, Ambarsari L, Nurcholis W. 2022. Variability total phenolic content and antioxidant activity of *Curcuma zanthorrhiza* and *C. aeruginosa* cultivated in three different locations in West Java, Indonesia. Biodiversitas 23: 1998-2003. *Curcuma zanthorrhiza* Roxb. and *C. aeruginosa* Roxb. are medicinal plants belonging to the Zingiberaceae family, which contained phenolic compounds. Phenolic is one of the antioxidant compounds. This study aimed to investigate variation on the total phenolic and antioxidant activity of three *C. zanthorrhiza* varieties and twenty *C. aeruginosa* genotypes cultivated in three different locations in West Java, Indonesia. Total phenolic content was determined using the Folin-Ciocalteu reagent, whereas antioxidant activity was used spectrophotometry using DPPH method. The total phenolic content ranged from 2.403 to 7.539 mg GAE/ g DW) differed significantly among the *C. zanthorrhiza* and *C. aeruginosa* varieties/genotypes, with the highest concentration found in the Cursina 3 variety of *C. zanthorrhiza* in Cianjur. The antioxidant activity ranged from 2.443 to 14.960 µmol TE/g DW, with the maximum activity identified in the Cursina 2 variety of *C. zanthorrhiza* in the Bogor location. G1, G6, G13, and G16 were identified as stable genotypes for phenolic antioxidant production. Three *C. zanthorrhiza* cultivars demonstrated higher total phenolic content and antioxidant activity than all *C. aeruginosa* genotypes in all planting areas. *C. zanthorrhiza* cultivars are the most promising source of phenolic content and antioxidant activity and are thus recommended for mass plantation at suitable locations in West Java, Indonesia to maximize potential.

Keywords: Antioxidant activity, *Curcuma aeruginosa*, *Curcuma zanthorrhiza*, DPPH, phenolic

INTRODUCTION

Indonesia has a wide variety of medicinal plants, having between 2500 and 2700 plant species (Cahyaningsih et al. 2021). Rhizomes of some species within the Zingiberaceae family are frequently utilized as medicinal plants, two species of which are *Curcuma zanthorrhiza* Roxb. and *C. aeruginosa* Roxb. (Yuanadini et al. 2021). Several studies reported pharmacological activities from these plants, including anticancer, antidiabetic, antimicrobial, and antioxidant properties (Akarchariya et al. 2017; Nurcholis et al. 2017; Nurcholis et al. 2018; Fitria et al. 2019). This demonstrates the significance of these plants as potential Indonesian medicinal herbs.

Secondary metabolites, such as polyphenols influence the pharmacological activities of *C. zanthorrhiza* and *C. aeruginosa* rhizomes. Curcuminoid compounds consisting of curcumin, demethoxy curcumin, and bisdemethoxy curcumin are polyphenolic compounds that have been reported to be present in the rhizomes of *C. zanthorrhiza* and *C. aeruginosa* (Nurcholis et al. 2016a; Nurcholis et al. 2016b; Nurcholis et al. 2019; Atun et al. 2020; Nurcholis et al. 2020). Many studies have found polyphenols to be effective as antioxidants (Bahukhandi et al. 2018; Feduraev et al. 2019; Xiang et al. 2019), and as a result, evaluating polyphenol in samples will be excellent compared to antioxidant activity. These parameters have been used in several studies of *C. zanthorrhiza* and *C. aeruginosa*. Sandrasari et al. (2019) evaluated phenolic and antioxidant activity in rhizomes of five species of Zingiberaceae native to Indonesia with maximum identified results in *Zingiber officinale* followed by *C. longa*, *C. zedoaria*, *C. zanthorrhiza* and *C. aeruginosa*. Phenolic and antioxidant characteristics were also employed in other studies, such as the use of solar drier (Vitanti et al. 2016), identifying elite accessions (Khumaida et al. 2019; Rosidi 2020), and evaluating Indonesian Zingiberaceae herbs (Muflihah et al. 2021).

Plant genotype and environmental conditions and interactions between genotype x environment are essential factors affecting the productivity of medicinal plant secondary metabolites. Productivity secondary metabolites have been shown in several plants, including *C. longa* (Pal et al. 2020), *Solanum viarum* (Patel et al. 2022), *Andrographis paniculata* (Kalariya et al. 2021), and *Amelanchier alnifolia* (Lachowicz et al. 2017), which are...
affected by genotype x environment. According to Seno et al. (2020), C. zanthorrhiza varieties and C. aeruginosa genotypes grown at different locations were affected by photosynthetic parameters and rhizome productivity. Photosynthesis is an essential parameter for producing primary plant metabolites which are precursors in the biosynthesis of secondary metabolites in plants (Nocchi et al. 2020).

We suspect that C. zanthorrhiza and C. aeruginosa cultivated at different locations will also affect the content of secondary metabolites, one of which is phenolic compounds. Until present, research on the total phenolic content and antioxidant activity of C. zanthorrhiza varieties and C. aeruginosa cultivated in different locations has not been reported. Therefore, this study aimed to investigate total phenolic content and antioxidant activity in C. zanthorrhiza and C. aeruginosa extract as well as identified stable genotype that cultivated in three different locations in West Java, Indonesia.

MATERIALS AND METHODS

Study area

Plant materials used in the study were collected from three locations in West Java, Indonesia from October 2019 to July 2020 (Table 1).

Plant materials

Curcuma zanthorrhiza varieties namely Cursina 1, Cursina 2, and Cursina 3 (C1-C3) used in the study were received from Indonesian Spices and Medicinal Crops Research Institute, Bogor, West Java, Indonesia. The C. aeruginosa genotypes (G1-G20) were obtained from Agricultural Biochemistry Laboratory, Department Biochemistry, IPB University, Bogor, Indonesia. All C. zanthorrhiza and C. aeruginosa were grown under identical conditions, except for the cultivated sites, and harvested at the same time in the same regions. Three samples of each C. zanthorrhiza variety and C. aeruginosa genotype from each site were evaluated in this work. All of the samples’ rhizomes were broken into small pieces and air-dried before being processed into a powder.

Procedures

Plant extraction

All dried rhizomes were extracted utilizing the sonication with maceration procedure, as described in Calvindi et al. (2020), with minor modifications. Rhizome sample of 4 g was extracted with 80% ethanol (20 mL) and then sonicated using Branson 1510 for 30 min. The solution was macerated for 3 h at room temperature in dark condition. Then, the solution was filtered using filter paper. All of the extraction steps were done again three times. Finally, the supernatant was concentrated or adjusted to a final concentration extract of 0.2 g/mL and this extract was used for the evaluation of total phenolic content and antioxidant activity.

Total phenolic content

The determination was performed using the previously described 2,2-diphenyl picrylhydrazyl (DPPH) technique (Nurcholis et al. 2021). The amount of antioxidant activity was expressed in micromol Trolox equivalents per g of dry weight (mg GAE/ g DW).

Data analysis

ANOVA was determined using the ExpDes package in the R program, followed by a Scott-Knot test (Ferreira et al. 2014). When p <0.05 is evaluated, considerable significant differences. The stability of samples was determined using additive main effect and multiplicative interaction (AMMI) with using PBSTAT-GE (www.pbstat.com). Visualization of genotype stability was used AMMI-2 biplot based on data of total phenolic content and antioxidant activity obtained from the pbstat.com on the GGE (genotype plus genotype-by-environment) menu.

Table 1. Information of experimental sites condition in Bogor, Cianjur, and Sukabumi, West Java

| Locations                                      | Latitude (S)  | Longitude (E) | Altitude (m asl.) | Rainfall (mm)  | Temp. (°C) |
|------------------------------------------------|---------------|---------------|-------------------|----------------|------------|
| Tropical Biopharmaca Research Center, IPB University, Dramaga, Bogor, West Java | 6°33'00.0"   | 106°43'12.0" | 141               | 424.6          | 26.4       |
| Pasir Sarongge, IPB University, Ciputri, Cianjur, West Java | 6°46'12.0"   | 107°03'00.0" | 1083              | 249.1          | 20.6       |
| Local farmer, Nagrak, Sukabumi, West Java       | 6°52'12.0"   | 106°48'00.0" | 493               | 321.1          | 17.6       |

Source: Meteorological, Climatological, and Geophysical Agency in Bogor, West Java, Indonesia (data monthly 2019-2020).
RESULTS AND DISCUSSION

Total phenolic contents

Total phenolic contents (TPC) in 20 genotypes of C. aeruginosa thimzone ranged from 2.403 to 5.118 mg/g GAE DW and 3 varieties of C. zanthorrhiza thimzone ranged from 4.871 to 7.539 mg/g GAE DW. The average TPC of C. aeruginosa and C. zanthorrhiza was the highest produced by C. aeruginosa and C. zanthorrhiza cultivated in the Bogor (3.872 mg/g GAE DW), followed by C. aeruginosa and C. zanthorrhiza that cultivated in Cianjur (3.728 mg/g GAE DW) and Sukabumi (3.651 mg/g GAE DW). The highest mean total phenolic content of C. aeruginosa based on its genotype was G14 (3.909 mg/g GAE DW) after comparison Cursina 1 (C1), Cursina 2 (C2), and Cursina 3 (C3). The maximum TPC of C. zanthorrhiza varieties was found in C3 cultivated in Cianjur, followed C2 and C3 in Bogor. C. zanthorrhiza varieties thimzone contained high TPC compared to the C. aeruginosa genotypes (Table 2).

Antioxidant activity

The antioxidant activity values of C. aeruginosa ranged from 2.443 to 6.794 μmol TE/g DW and C. zanthorrhiza ranged from 5.265 to 14.960 mol TE/g DW. Genotype G1 (5.547 μmol TE /g DW) has the highest mean value of antioxidant after comparison and Bogor (5.034 μmol TE/g DW) is the location with the highest antioxidant activity value compared to Sukabumi (4.951 μmol TE/g DW) and Cianjur (4.333 μmol TE/g DW). The highest antioxidant activity of C. zanthorrhiza varieties was found in C2 followed C1 and C3 cultivated in Bogor (Table 3).

Genotype stability

The results of the combined variance analysis of 20 genotypes of C. aeruginosa and 3 varieties of C. zanthorrhiza showed that the genotypes had a very significant effect with p-value less than 0.001 for TPC and antioxidant activity (Table 4). The p-value less than 0.001 for the total phenolic content and antioxidant activity indicated that there was variation in yield based on the genotype. Experimental replicates on antioxidant activity had a p-value of 0.011 and the interaction between genotype and environment had a significant effect with a p-value of 0.009 for antioxidant activity of DPPH. The interaction between genotype and environment, PC1 has p-value of 0.088 for total phenolic content and p-value of less than 0.001 for antioxidant activity.

The AMMI-2 biplot can be used to describe the genotype’s stability across all experimental sites by utilizing the confidence region of the ellipse with a center point of (0.0) and the first and second most significant interaction principal component (PCA) values. The contribution of the diversity of interaction effects on the total phenolic parameters and antioxidant activity that can be explained by the main component of the interaction, which is 100% with each component PC1 and PC2 on the total phenolic parameters were 67.1% and 32.9% (Figure 1) and the antioxidant activity parameters of components PC1 and PC2 were 89.1% and 10.9%, respectively (Figure 2).
Table 4. Analysis of variance for AMMI model of 20 genotypes of C. aeruginosa and 3 varieties of C. zanthorrhiza

| Source of variation       | DF | SS   | MS   | F     | P-value |
|---------------------------|----|------|------|-------|---------|
| Total phenolic content    |    |      |      |       |         |
| Environment               | 2  | 1,798| 0.899| 2.494 | 0.162   |
| Replication/Environment   | 6  | 2.163| 0.360| 0.417 | 0.866   |
| Genotype                  | 22 | 262.177| 11.917| 11.958 | <0.001* |
| Genotype x Environment    | 44 | 43.847| 0.996| 1.182 | 0.266   |
| PC1                       | 23 | 29.430| 1.279| 1.48  | 0.088   |
| PC2                       | 21 | 14.416| 0.686| 0.79  | 0.727   |
| Error                     | 132| 114.092| 0.864|       |         |

Antioxidant activity

| Source of variation       | DF | SS   | MS   | F     | P-value |
|---------------------------|----|------|------|-------|---------|
| Environment               | 2  | 19.981| 9.990| 8.585 | 0.470   |
| Replication/Environment   | 6  | 69.863| 11.643| 2.863 | 0.011   |
| Genotype                  | 22 | 959.169| 43.598| 6.015 | <0.001* |
| Genotype x Environment    | 44 | 309.140| 7.025| 1.727 | 0.009   |
| PC1                       | 23 | 275.349| 11.971| 2.94  | <0.001* |
| PC2                       | 21 | 33.790| 1.609| 0.4   | 0.991   |
| Error                     | 132| 536.813| 4.067|       |         |

Note: *Significance at p <0.001

Stable genotypes are presented by the AMMI-2 biplot, which is the genotype inside the circle (ellipse) (Figure 1). There are 8 genotypes namely G1, G2, G3, G4, G6, G12, G13, and G16 based on TPC, while the specific genotype for total phenolic parameters, C. aeruginosa genotype G7 is specific for the Sukabumi area, G18 is specific for the Bogor area and G5 and G15 were specific for the Cianjur area. There are 6 stable genotypes with antioxidant activity parameters that is G1, G5, G6, G13, G16, and G17 (Figure 2). Specific genotypes based on antioxidant activity parameters in the Cianjur area were G7, G8, G15, G18, and G19. The C. zanthorrhiza variety C1 is a specific variety for the Bogor area and C. aeruginosa genotypes G9 and G13 are the specific genotypes for the Sukabumi area.

Discussion

Phenolics are key secondary metabolites that are effective antioxidants (Zeb 2020). Antioxidant qualities in plants can lower the risk of a variety of diseases, such as cancer (Rôleira et al. 2015), diabetes (Lin et al. 2016), hypercholesterolemia (Wang et al. 2012), and other cardiovascular disorders (Olas 2017). As a result, there is a strong demand for plant materials with high phenolic content and antioxidant capacities (Machu et al. 2015). C. zanthorrhiza showed a greater total phenolic content and antioxidant activity than C. aeruginosa (Tables 2 and 3). These results are consistent with the findings of previous studies (Nurcholis et al. 2012; Sandrasari et al. 2019). The C2 variety of C. zanthorrhiza cultivated in Bogor produced the highest TPC (7.304 mg GAE/g DW) and antioxidant activity (14.960 µmol TE /g DW) compared to other plant samples. C. zanthorrhiza variety C2 in Bogor, West Java, Indonesia, has great promise as a source of antioxidant phenolic commercially.

Environmental factors influenced the total phenolic content and antioxidant activity in plants studied in this work. Bogor with the highest rainfall and temperature conditions showed maximum phenolic antioxidant contents than Cianjur and Sukabumi. This finding is consistent with prior studies on Salvia officinalis, which have shown that
rainfall and growth environment temperature affected phenolic and antioxidant production (Generalić Mekinić et al. 2019). Additionally, various studies have demonstrated that altitude affects the antioxidant phenolic content of plants (Cirak et al. 2022). The low altitude was found in Bogor, which identified the plant's highest phenolic and antioxidant production. Previous studies on Achilllea collina found that the production of antioxidant phenolics increased when the plants were cultivated in environments with a high altitude (Giorgi et al. 2010). This research indicated that the phenolic antioxidant content in C. zanthorrhiza and C. aeruginosa plants is influenced by altitude, rainfall, and temperature conditions of cultivated area locations.

Evaluating genotypes/cultivars in different environments is crucial in a plant breeding program. The results presented the genotypes/varieties (C. zanthorrhiza and C. aeruginosa) main effects were significant (p < 0.001) but not significant in environmental as well as the genotype-by-environment interaction for total phenolic content and antioxidant activity (Table 4). This finding differs from our prior findings on rhizome productivity and photosynthetic rate, which showed that genotype and environment interacted to influence C. zanthorrhiza and C. aeruginosa samples (Seno et al. 2020). Both the total phenolic and antioxidant characteristics of the C2 variety indicated a specific location in Bogor. Numerous stable C. aeruginosa genotypes, namely G1, G6, G13, and G16, suggest a high potential for new varieties to be generated through plant breeding programs (Elias et al. 2016; Pour-Aboughadareh et al. 2019).

In conclusion, the C. zanthorrhiza varieties cultivated in Bogor have the highest total phenolic content and antioxidant activity. Cursina 2 variety of C. zanthorrhiza has emerged as one of the most promising cultivars, and hence mass planting in their ideal site is recommended. The stable genotypes for phenolic antioxidant production were G1, G6, G13, and G16. These genotypes were potentially to develop a new variety through plant breeding programs.

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