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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. (Yuandani 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, dimethoxy 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 have 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 Folin-Ciocalteu spectrophotometric technique described by Batubara et al. (2020) with minor modifications was used to estimate the total phenolic content (TPC) by using a nano-spectrophotometer (SPECTROstarNano BMG LABTECH). TPC was reported as mg of gallic acid equivalents per g of dry weight (mg GAE/ g DW).

Antioxidant activity

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 (μmol TE/ 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* rhizome ranged from 2.403 to 5.118 mg/g GAE DW and 3 varieties of *C. zanthorrhiza* rhizome 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 rhizome 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 2. Total phenolic content 20 genotypes of *C. aeruginosa* and 3 varieties of *C. zanthorrhiza* in the three different locations

Genotype/ variety	Bogor	Sukabumi	Cianjur	Mean
	Total phenolic content (mg/g GAE DW)			
G1	3.508b	3.882c	3.596c	3.662b
G2	3.365b	3.024c	3.310c	3.233b
G3	3.183b	2.642c	2.944c	2.923b
G4	2.867b	2.914c	2.789c	2.857b
G5	2.643b	2.572c	4.137c	3.118b
G6	4.084b	3.384c	3.884c	3.784b
G7	3.312b	4.251c	3.304c	3.622b
G8	3.062b	3.399c	3.554c	3.338b
G9	3.805b	3.981c	2.743c	3.510b
G10	4.196b	3.359c	3.171c	3.576b
G11	4.058b	3.353c	3.024c	3.478b
G12	3.290b	3.465c	3.145c	3.300b
G13	3.457b	2.850c	2.982c	3.096b
G14	2.830b	5.118b	3.778c	3.909b
G15	3.035b	2.565c	3.334c	2.978b
G16	3.207b	2.948c	2.827c	2.994b
G17	3.659b	2.403c	3.342c	3.135b
G18	3.543b	2.611c	2.887c	3.014b
G19	2.755b	4.100c	3.770c	3.541b
G20	3.882b	3.433c	3.065c	3.460b
C1	6.780a	6.418a	5.690b	6.296a
C2	7.304a	4.871b	6.596b	6.257a
C3	7.236a	6.422a	7.539a	7.066a
Mean	3.872A	3.651A	3.728A	

Note: a-c: means within a column with different letters are significantly different (Scoot-Knot test, p <0.05), A: means within a row with different letters are significantly different (Scoot-Knot test, p <0.05)

Table 3. Antioxidant activity 20 genotypes of *C. aeruginosa* and 3 varieties of *C. zanthorrhiza* in three different locations

Genotype/ variety	Bogor	Sukabumi	Cianjur	Mean
	Antioxidant activity (µmol TE/g DW)			
G1	6.794b	4.692a	5.155b	5.547c
G2	3.234b	4.895a	3.562b	3.897c
G3	4.342b	3.393a	5.562b	4.432c
G4	4.014b	3.652a	3.404b	3.690c
G5	4.353b	3.133a	3.822b	3.769c
G6	3.777b	4.681a	4.647b	4.368c
G7	3.698b	4.014a	5.008b	4.240c
G8	3.675b	3.483a	4.844b	4.001c
G9	2.997b	4.376a	3.652b	3.675c
G10	3.573b	4.093a	3.347b	3.671c
G11	2.997b	4.816a	5.325b	4.379c
G12	2.443b	3.816a	2.850b	3.037c
G13	2.929b	3.438a	3.178b	3.182c
G14	4.150b	4.455a	3.912b	4.172c
G15	3.856b	4.025a	5.652b	4.511c
G16	3.370b	2.861a	3.980b	3.404c
G17	2.477b	3.924a	4.229b	3.543c
G18	3.178b	3.573a	4.613b	3.788c
G19	3.347b	3.551a	4.669b	3.856c
G20	4.816b	3.799a	3.765b	4.127c
C1	14.641a	5.265a	7.005b	8.970b
C2	14.960a	7.183a	9.953a	10.699a
C3	12.155a	8.533a	11.729a	10.805a
Mean	5.034A	4.951A	4.333A	

Note: a-c: means within a column with different letters are significantly different (Scoot-Knot test, p <0.05), A: indicates that means within a row with different letters are significantly different (Scoot-Knot test, p <0.05)

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	1.152	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					
Environment	2	19.981	9.990	0.858	0.470
Replication/Environment	6	69.863	11.643	2.863	0.011
Genotype	22	959.169	43.598	6.205	<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	4.0667		

Note: *Significance at *p* <0.001

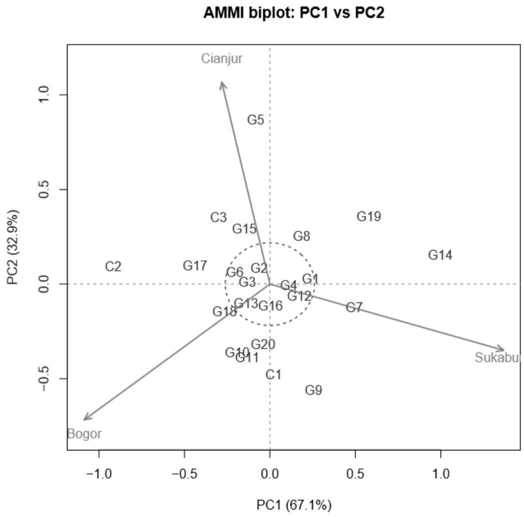

Figure 1. Genotype stability of total phenolic content 20 genotypes of C. *aeruginosa* and 3 varieties of C. *zanthorrhiza*

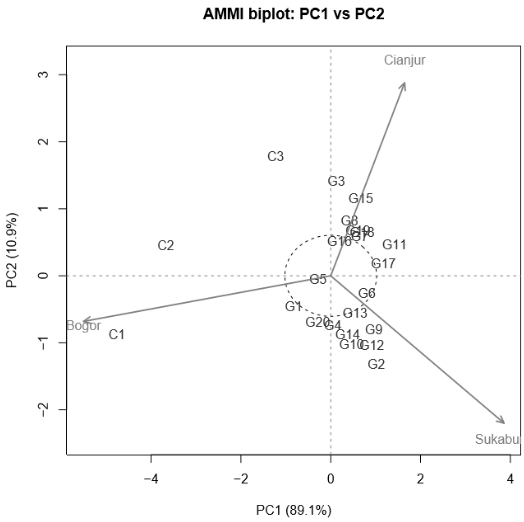

Figure 2. Genotype stability of antioxidant activity 20 genotypes of C. *aeruginosa* and 3 varieties of C. *zanthorrhiza*

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 (Roleira 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 studied (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 *Achillea 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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