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Variability of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin Contents in Ethanolic Extract from Ten *Curcuma aeruginosa* Roxb. Cultivated in West Java, Indonesia

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Ten accessions of *Curcuma aeruginosa* Roxb. were grown under the same environment in Bogor, West Java, Indonesia was investigated for curcumin, demethoxycurcumin and bisdemethoxycurcumin contents using HPLC. No significant variations were observed among *C. aeruginosa* accessions for all the studied compounds. According to the results, curcumin content (mg/100 g extract) varied between 1.08 in accession Kulonprogo to 3.19 in accession Gunung Kidul. The accessions Muara Bungo and Kulonprogo had the highest (1.66) and lowest (0.51) levels of demethoxycurcumin (mg/100 g extract). Also, bisdemethoxycurcumin content varied from 0.05 to 0.49 mg/100 g extract in accessions Ciampea Bogor and Purworejo, respectively. Finally, curcuminoid contents (mg/100 g extract), accumulation of curcumin, demethoxycurcumin and bisdemethoxycurcumin contents, ranged from 1.64 to 5.05 in accessions Kulonprogo and Gunung Kidul, respectively. Hierarchical cluster analysis showed that 10 accessions could be divided into three groups. Group 1 composed of Gunung Kidul, Muara Bungo, Pakem and Beringharjo accessions. These accessions characterized by high of curcumin, demethoxycurcumin, and bisdemethoxycurcumin contents. Group 2 formed two accessions (Klewer and Purworejo) that showed the high only bisdemethoxycurcumin content. Accessions of Kulonprogo, Losari Cirebon, Madura and Ciampea Bogor, in group 3, characterized by low curcumin, demethoxycurcumin and bisdemethoxycurcumin contents. In this study, Gunung Kidul, Muara Bungo, Pakem and Beringharjo accessions have a high in curcuminoid content that showed a high potential for use in future plant breeding programs.

Keywords: Bisdemethoxycurcumin, *Curcuma aeruginosa* Roxb., Curcumin, Curcuminoid, Demethoxycurcumin.

INTRODUCTION

Curcuma aeruginosa Roxb., from the Zingiberaceae family and commonly known as "temu hitam or temu ireng" in Indonesia, was widely grown around in Indonesia [1]. The rhizomes are part of plant that is commonly used as a traditional medicine for treating dyspepsia, flatulence, inflammation and microbe infections [2,3]. Several pharmacological activities of *Curcuma aeruginosa* rhizome have been reported in recent years. Essential oils of *C. aeruginosa* have been demonstrated as antimicrobial activity [2,4]. Other studies showed some biological activities from the rhizome of *Curcuma aeruginosa*, such as uterine relaxant [5], anti-androgenic [6] and improvement number of thrombocytes for dengue fever treatment [7].

One compound of *C. aeruginosa* that is responsible for pharmacological activities belongs to the group of secondary metabolites known as curcuminoids [8]. Curcuminoids, a diaryl-heptanoids compound, are the significant pharmacological bioactive compounds in Curcuma species such as *C. longa*, *C. zanthorrhiza* and *C. aeruginosa* [2,9,10]. The main compound in this group are curcumin [(1*E*,6*E*)-1,7-*bis*(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione], demethoxycurcumin [(1*E*,6*E*)-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione] and bisdemethoxycurcumin [(1*E*,6*E*)-1,7-*bis*(4-hydroxyphenyl)hepta-1,6-diene-3,5-dione] [11].

Recently several studies have shown that curcuminoids have antiageing [12], antioxidant, antimicrobial and anti-

inflammatory [13], antiprotozoal [14], antivenom [15], antimalarial [16], antitumor [17], antiangiogenic [18] and anticancer [19] activities. Because of the valuable pharmacological properties of curcuminoids, evaluation of curcumin, demethoxycurcumin and bisdemethoxycurcumin of *C. aeruginosa* diversity in every region can guide to access appropriate selection accessions for a breeding program or direct use in commercial scale.

The metabolism and accumulation of secondary metabolites in the medicinal plant influenced by genetic and environmental factors [20]. Previous studies [8,10,21] showed the existence of different curcuminoids contents and pharmacological activities of *Curcuma aeruginosa* accessions from different location of Indonesia. The information of curcuminoids contents of *Curcuma aeruginosa* accessions were still limited as they cultivated in the same environmental conditions.

The food and pharmaceutical product industry need genetically genotypes that are uniform to get the same accumulation metabolites productivity through plant breeding program of agricultural biochemistry. In this study, the medicinal plant of *C. aeruginosa* was selected because it is one of the rhizomes medicinal plant which is popular in traditionally but has limited usage in the industry. This work was carried out to document the evaluation of ten *Curcuma aeruginosa* accessions and their curcuminoids contents. In order to mark the best source of curcuminoids contents of ten *C. aeruginosa* accessions, which were grown in the same conditions in West Java, these metabolites have been investigated. Furthermore, it is beneficial to discover *C. aeruginosa* accessions with the best performance for curcuminoids productivity to be used as preliminary genotypes for plant breeding program of agricultural biochemistry.

EXPERIMENTAL

Ten rhizomes of *Curcuma aeruginosa* accessions were obtained from result selection of previous research, samples collected from different regions in East Java, Central Java, Yogyakarta, West Java and Jambi in February 2015, based on metabolites contents and biological activities [10,21-23]. Ten *C. aeruginosa* accessions were namely given codes with KL (Klewer), PK (Pakem), BH (Beringharjo), GK (Gunung Kidul), KP (Kulonprogo), PW (Purworejo), MD (Madura), LC (Losari Cirebon), CB (Ciampea Bogor) and MB (Muara Bungo). The standards of curcumin, demethoxycurcumin and bisdemethoxycurcumin were purchased from ChromaDex Inc. (Santa Ana, CA, USA). Other solvents used analytic grade and obtained from Merck (Darmstadt, Germany).

The curcuminoids contents of samples were determined using high performance liquid chromatography (HPLC, Shimadzu LC-20A series, Japan). HPLC equipped with Shimpack VP-ODS C18 column (150 mm × 4.6 mm identification, 4.6-micrometer particle size) and diode array UV detector (Shimadzu, Tokyo, Japan).

Plant materials and extractions: The cultivation was performed in a completely randomized design in triplicate replication in December 2015. Ten *C. aeruginosa* accessions were grown in the same conditions on a latosol soil (pH 4.5-5, N of 0.15 % and organic C of 1.52 %) in the Biopharmaca Conservation and Cultivation Station, IPB University, West Java, Indonesia (6º32′25.47″ N and 106º42′53.22″ E, at 142.60 m altitude). The plant spacing was 50 cm × 50 cm. After 2 weeks, the soil was treated with 1 kg cow manure per planting hole then the rhizome accessions planted. The rhizomes accessions were harvested in August 2016 (9 months after planting). The fresh rhizome samples were harvested then washed, cut, dried and crushed to a powder (100 mesh). The powder samples (25 g) macerated with 70 % ethanol (250 mL) at room temperature during 24 h. Then, the sample solution was filtered using a Whatman filter paper No. 41. The sample extract was obtained by evaporation (BUCHI, R-250, Switzerland) of sample solution at 50 ºC. The extracts ranged from 3.63 to 7.36 % were then used in curcuminoids determinations.

Analysis of curcuminoids contents: The ethanolic extracts of *C. aeruginosa* accessions were used to determine curcumin, demethoxycurcumin and bisdemethoxycurcumin (curcuminoids) contents with using HPLC method with slight modification [24]. Before injected to HPLC (20 µL), sample extract (50 mg) or standards was sonicated with methanol (10 mL) for 1 h at room temperature and filtered through a 0.45 µm Nylon filter. Acetonitrile (A), acetic acid and water (B) were used as a solvent. The elution profile was 0-30 min with 40 %-70 % A; 30-40 min with 100 % B. The temperature set at ambient and 1 mL/min flow rate. The quantitation of curcuminoids contents was monitored at 425 nm by comparing the areas with standard curcumin, demethoxycurcumin and bisdemethoxycurcumin. The concentration of curcumin, demethoxycurcumin and bisdemethoxycurcumin (curcuminoids) contents were expressed as mg/100 g extract.

Statistical analysis: Results were expressed as the mean from three biological replicates ± SEM. ANOVA was performed using RStudio version 1.1.456. MetaboAnalystR was used for similarity analysis of curcuminoids contents from *Curcuma aeruginosa* accessions using Hierarchical cluster analysis with used Euclidean distances and Ward clustering [25].

RESULTS AND DISCUSSION

The results of curcumin contents of ethanolic extracts of *Curcuma aeruginosa* accessions are summarized in Fig. 1. The values of curcumin contents found in different evaluated *C. aeruginosa* accessions ranged from 1.08 to 3.19 mg/100 g extract, not significantly different at $p < 0.05$, demonstrated by Kulonprogo and Gunung Kidul accessions, respectively. The previous study has been analyzed the curcumin contents which samples from different regions and found much higher values for *C. aeruginosa* (0.01-1.45 %, b/b) compared to those found in this study [8]. For another *Curcuma* sp. several authors also reported higher values as 2.3-10.9 % in turmeric (*C. longa* L.) [26] and 24.7 to 54.1 mg/g in Java turmeric (*C. zanthorrhiza*) [9] that also belongs to the Zingiberaceae family.

According to ANOVA, demethoxycurcumin contents of all accessions showed did not differ significantly at $p < 0.05$ (Fig. 2). The Muara Bungo and Gunung Kidul accessions demonstrated high of demethoxycurcumin contents. These accessions remained in the class with higher contents, providing 1.66 and 1.58 mg/100 g extract, respectively. The demethoxycurcumin contents of *C. aeruginosa* in the sample which collected from different geographic showed highest with ranged value of 0.01 to 0.47 %, compared to those reported in this work [8].

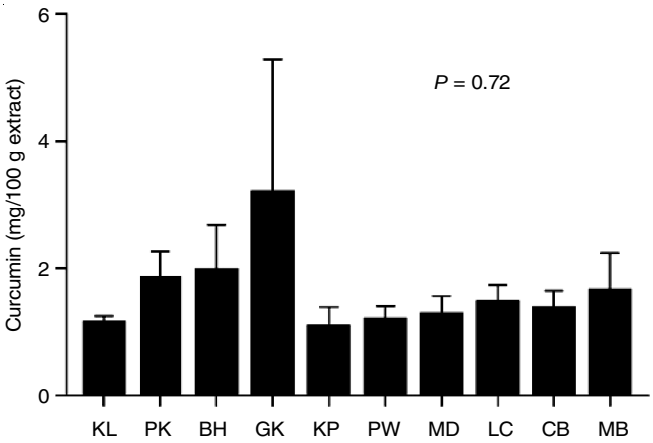

Fig. 1. Curcumin content in ethanolic extract of different accessions of *C. aeruginosa*. Data are mean of a minimum three biological replicates (± SEM). *P* value for the analysis of variance

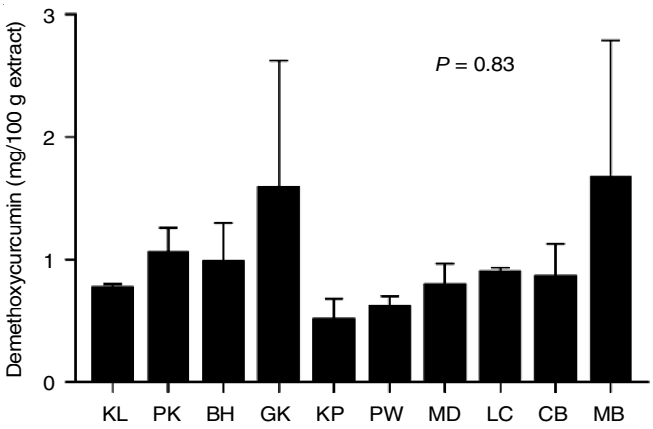

Fig. 2. Demethoxycurcumin content in ethanolic extract of different accessions of *C. aeruginosa*. Data are mean of a minimum three biological replicates (± SEM). *P* value for the analysis of variance

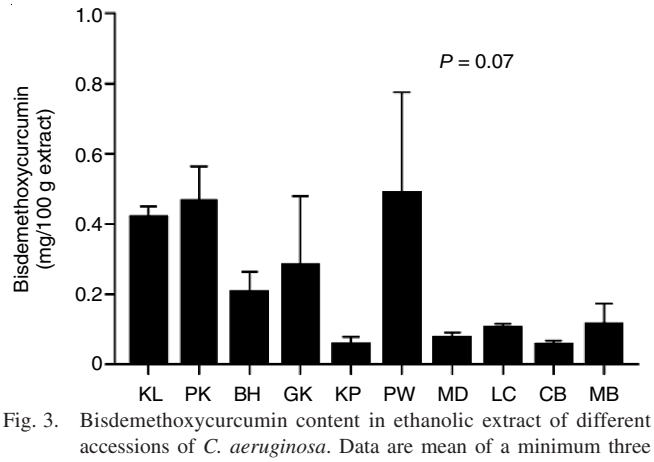

Fig. 3. Bisdemethoxycurcumin content in ethanolic extract of different accessions of *C. aeruginosa*. Data are mean of a minimum three biological replicates (±SEM). *P* value for the analysis of variance

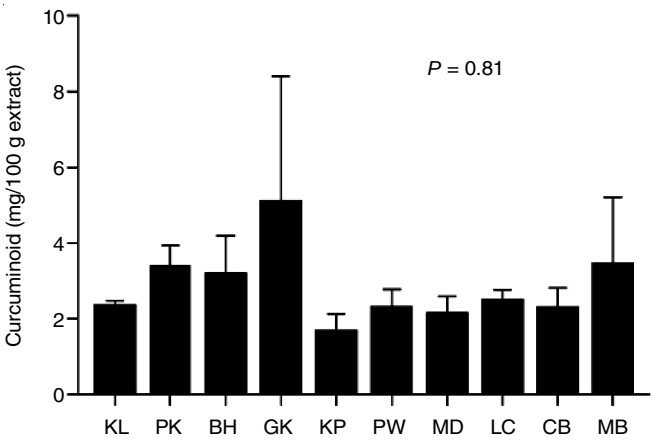

Fig. 4. Curcuminoid content in ethanolic extract of different accessions of *C. aeruginosa*. Data are mean of a minimum three biological replicates (±SEM). *P* value for the analysis of variance

Demethoxycurcumin from other *C. aeruginosa* that collected from Yogyakarta, Surabaya and Solo, Indonesia, ranged between 0.01 and 0.02 % [27].

Bisdemethoxycurcumin content was not varied significantly ($p < 0.05$) in different accessions of *C. aeruginosa* studied, as seen in Fig 3. Purworejo, Pakem and Klewer accessions showed highest of bisdemethoxycurcumin content with values of 0.49, 0.47and 0.42 mg/100 g extract, respectively. While other accessions was low detected with value of < 0.30 mg/100 g extract. This result similar to the previous report [27], which showed no detection of bisdemethoxycurcumin in ethanol extract from a different sample of *C. aeruginosa*. The results were, however, lower than that of bisdemethoxycurcumin compound in turmeric (*C. longa* L.) (2.90-9.10 mg/100 g sample) and Java turmeric (*C. zanthorrhiza*) (0.80-1.00 mg/100 g sample) [28]. Generally, it can be said that *C. aeruginosa* has the lowest bisdemethoxycurcumin content as shown in this study.

The amounts of curcumin, demethoxycurcumin and bisdemethoxycurcumin compounds were expressed as curcuminoids contents in ethanolic extract of *C. aeruginosa* accessions as shown in Fig. 4. Statistical analysis of curcuminoids contents showed no significant difference ($p < 0.05$) between the ten *C. aeruginosa* accessions studied. Curcuminoids in *Curcuma aeruginosa* ranged from 1.64 to 5.05 (mg/100 g extract) between the ten accessions studied. At accessions selected, the highest amount of curcuminoid (5.05 mg/100 g extract) was observed in accession Gunung Kidul, while the lowest was observed in accession Kulonprogo (1.64 mg/100 g extract). In *Curcuma aeruginosa* that collected from different regions in Indonesia showed highest curcuminoids contents (0.01 % to 1.95 %) compared to those recorded in this work [8]. The curcuminoids compounds were the major diarylheptanoid which contained in *Curcuma* species, such as *C. domestica*, *C. zanthorrhiza* and *C. aeruginosa* [29,30]. Of the *Curcuma* sp, the curcuminoid content was highest in *C. domestica* (2.9-9.1 %) when compared with *C. zanthorrhiza* (0.8-1.0 %) and *C. aeruginosa* (0.02-0.03 %) [27,28]. Of Curcuma species, several factors influenced the change of curcuminoids contents including the cultivation technique, varieties, source and location [24,31,32].

Because this study used in the same environment location with no different cultivation methods, therefore the varieties and source factors may have affected the unusual accumulation of curcuminoids contents in sample *C. aeruginosa* rhizome. Thus, the recorded variations with their curcuminoids contents were closely related to genetic factors of *Curcuma aeruginosa* accessions as demonstrated by Gilani *et al*. [33] and Arya *et al*. [34] in the case of curcuminoids contents on the rhizomes of *C. amanda* and *C. longa*, respectively.

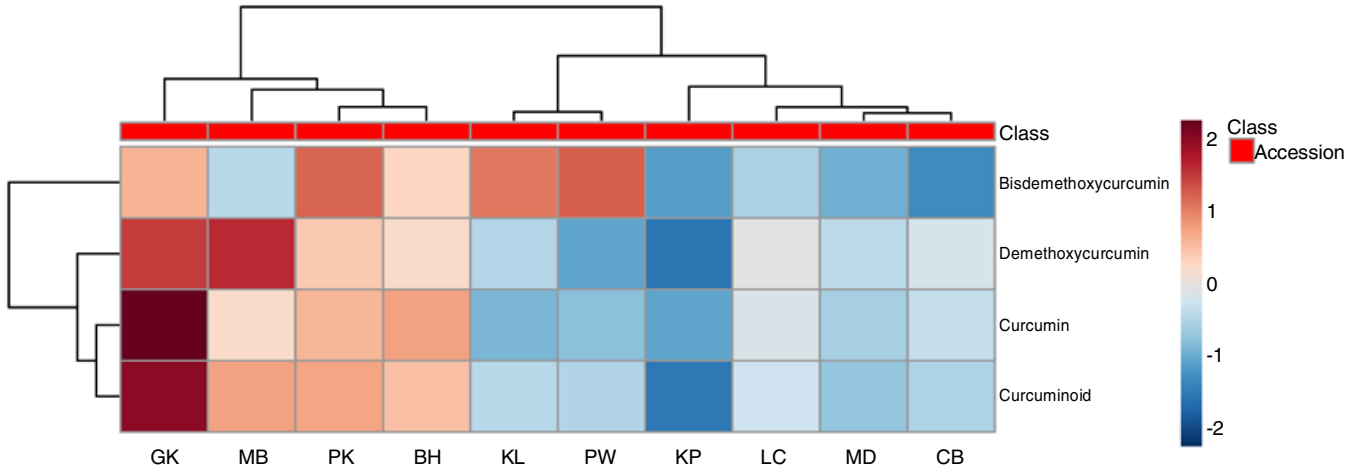

Fig. 5. Heat map and hierarchical clusters (HCA) of two-dimensional relationships among accessions of *C. aeruginosa* and metabolite of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and curcuminoid. The values of metabolite contents per accession, which has been log transformation and auto-scaling, are shown in the heat map on blue (negative) to red (positive) scale. Heat map and HCA of accessions were according to Euclidean distance and Ward clustering

Hierarchical cluster (HCA) based on metabolite of curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcuminoid among *C. aeruginosa* accessions were studied. Curcuminoids profile showed remarkable differences among *C. aeruginosa* accessions studied (Fig. 5). The heat map of two-dimensional, a dendrogram, show that 10 *Curcuma aeruginosa* accessions can be clustered into three clusters. In clusters, the similarity and curcuminoid diversity can be displayed within accessions. The first cluster (I) is compact showing high curcuminoid (curcumin, demethoxycurcumin and bisdemethoxycurcumin) contents, which composed four accessions such as Gunung Kidul, Muara Bungo, Pakem and Beringharjo. The second cluster (II) is formed of Klewer and Purworejo accessions, it is less compacted indicating lowest curcumin, demethoxycurcumin and curcuminoid, but higher bisdemethoxycurcumin than the first cluster. Cluster III was composed of four accessions: Kulonprogo, Losari Cirebon, Madura and Ciampea Bogor. These accessions were characterized by low curcuminoids contents such as curcumin, demethoxycurcumin and bisdemethoxycurcumin compared with cluster I. Of the two-dimensional HCA, the Gunung Kidul, Muara Bungo, Pakem and Beringharjo can be suggested as accessions selected in *Curcuma aeruginosa* studied for breeding program with the objective of improvement to produce a high of curcuminoids especially for curcumin, demethoxycurcumin and bisdemethoxycurcumin compounds.

Conclusion

The accessions of Gunung Kidul, Muara Bungo, Pakem and Beringharjo produced high metabolite of curcuminoids. Therefore, for commercial scale, these accessions selected for future breeding programs that would produce in high of the curcumin, demethoxycurcumin and bisdemethoxycurcumin compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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