Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Biochemistry

ISSN 1815-9923
DOI: 10.3923/ajb.2016.142.148

Research Article

Variability of Total Phenolic and Flavonoid Content and Antioxidant Activity Among 20 *Curcuma aeruginosa* Roxb. Accessions of Indonesia

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Abstract

Objective: *Curcuma aeruginosa* Roxb. is wild-growing rhizome herb in Indonesia with interesting pharmacological activities. This study evaluated the variability of total phenolic, total flavonoid contents and their antioxidant activities of 20 Indonesian accessions of *C. aeruginosa*. **Methodology:** Total phenolic and flavonoid contents were determined by spectrophotometrically with the Folin-Ciocalteu and colorimetric method, respectively. Antioxidant activity was determined by inhibition using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. **Results:** The total phenolic and flavonoid contents and inhibition of DPPH in 20 accessions of *C. aeruginosa* were found to be in the range 23.13±2.92 to 78.59±12.23 mg TAE g^{-1}, 7.65±2.18 to 21.71±3.00 mg QE g^{-1} and 0.34±1.46 to 19.59±1.67%, respectively. Hierarchical cluster analysis from the 20 accessions of *C. aeruginosa* falls into 3 groups. Group I consisted of 12 accessions i.e., CB, KP, SH, WG, PW, KN, MD, PK, PT, GD, KL and MB. Group II consisted of 7 accessions i.e., GK, LC, KA, SG, PR, KD and BH. Group III consisted of 1 accessions i.e., NW. **Conclusion:** It is concluded that there is significant variability ($p<0.05$) in total phenolic and total flavonoid contents and DPPH scavenging activity of different accessions of *C. aeruginosa*. Evaluation of the performed on individual accession in the same location is required to determine variability in phytochemical composition of *C. aeruginosa*.

Key words: Temu hitam, phenolic, flavonoid, antioxidant, accessions

Received: March 19, 2016 Accepted: April 01, 2016 Published: April 15, 2016

Citation: Waras Nurcholis, Nurul Khumaida, Muhamad Syukur and Maria Bintang, 2016. Variability of total phenolic and flavonoid content and antioxidant activity among 20 *Curcuma aeruginosa* Roxb. accessions of Indonesia. Asian J. Biochem., 11: 142-148.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Curcuma aeruginosa Roxb. is commonly called "Temu hitam" in Indonesia, it belongs to the family Zingiberacea[1]. It has been identified as one of the popular traditional medicinal rhizomatous herbs that grow in the wild in Indonesia. The rhizome of *C. aeruginosa* has been reported to possess many interesting pharmacological activities such as antioxidant[2,3], anti-androgenic[4], antimicrobial[5, 6], antiviral for dengue fever[7] and HIV-1[8], anti-inflammation[9] and anticancer[10]. The phytochemical contents of the rhizome of *C. aeruginosa*, which have been investigated are tannins, saponins and triterpenoids[3], flavonoids, phenolics, alkaloids, steroids and tannins[5,11], alkaloids, polyphenols, tannins, saponins, flavonoids, monoterpenes, sesquiterpenes, steroids and kuinon[7], curcuminoids[12], germacrone[4], terpenoids, sterols, organic acids, fatty acids and sugars[13].

The *C. aeruginosa* qualities as medicinal plant material if their quality is standardized. Plant breeding can be an alternative method to produce quality and standardized materials of *C. aeruginosa*. Genetic variability is essential to the success of a breeding program[14]. In addition, medicinal plant conservation efforts are required to promote accession[15]. Therefore, a wide genetic variability of promoting accession is crucial for the success of breeding program of *C. aeruginosa*. The focus of the study is to determine the variability of total phenolic, total flavonoid contents and antioxidant activity of 20 Indonesian accessions of *C. aeruginosa*.

MATERIALS AND METHODS

Plant material and sample preparation: A total of 20 samples of *C. aeruginosa* were collected from different natural regions of Indonesia. Table 1 shows sampling locations and their geographical coordinates. Identification of specimen samples were confirmed by the Biopharma Research Center, Bogor Agricultural University, Indonesia. Fresh rhizome 20 samples of *C. aeruginosa* accessions were washed with water, cut into small pieces and dried for 5 days in the sun (moisture content, <10%) and then ground into powder with size of 100 mesh. The extraction was carried out using solvent maceration method with 70% (v/v) ethanol[3]. Briefly, 100 g powder samples were macerated with 1000 mL of 70% (v/v) ethanol at room temperature and for a period of 24 h and then filtered with type 4 Whatman filter paper. The whole process was repeated once (1×24 h). The crude extract was concentrated by evaporation (BUCHI, R-250, Switzerland) at ±50°C. These extracts (yield range, 7.92-19.71%) were then used in later experiments.

Total phenolic contents: The total phenolic content of extracts was determined according the Folin-Ciocalteu method[16] with slight modification. Briefly, 1 mL extracts were mixed with 9 mL of deionized water and 1 mL of the Folin-Ciocalteu's reagent. After 5 min, the solution was mixed with 10 mL of 7% (w/v) Na_2CO_3 and diluted to 25 mL with deionized water. After incubation for 90 min at room temperature, the absorbance at 750 nm was measured with

Table 1: Location of different accessions of *Curcuma aeruginosa* from different regions of Indonesia

Accession ID	Region	Province	Latitude (S)	Longitude (E)	Altitude (m)
MD	Madura	East Java	7°02'48.90"	112°43'47.32"	4
KD	Kediri	East Java	7°50'39.52"	111°53'54.93"	489
PR	Ponorogo	East Java	7°51'51.47"	111°28'11.78"	106
PT	Pacitan	East Java	8°11'59.56"	111°06'13.34"	7
NW	Ngawi	East Java	7°29'52.21"	111°09'22.78"	345
KA	Karanganyar	Central Java	7°39'49.37"	111°08'01.93"	1113
SG	Sragen	Central Java	7°24'22.14"	111°07'12.84"	90
GD	Gede-Solo	Central Java	7°34'08.83"	110°49'54.53"	95
KL	Klewer-Solo	Central Java	7°35'05.66"	110°49'45.38"	96
SH	Sukoharjo	Central Java	7°44'41.62"	110°52'41.14"	111
WG	Wonogiri	Central Java	7°57'22.83"	110°59'37.51"	378
PW	Purworejo	Central Java	7°44'25.35"	110°01'59.00"	56
KN	Kendal	Central Java	7°00'55.14"	110°16'05.98"	78
PK	Pakem	Yogyakarta	7°39'55.46"	110°25'11.30"	424
BH	Beringharjo	Yogyakarta	7°47'56.40"	110°22'01.56"	115
KP	Kulonprogo	Yogyakarta	7°56'25.03"	110°14'20.30"	20
GK	Gunung Kidul	Yogyakarta	7°58'04.87"	110°36'09.67"	180
LC	Losari-Cirebon	West Java	6°48'17.09"	108°48'06.04"	1
CB	Ciampea-Bogor	West Java	6°32'35.89"	106°41'22.41"	148
MB	Muara Bungo	Jambi	1°37'00.61"	102°22'16.28"	65

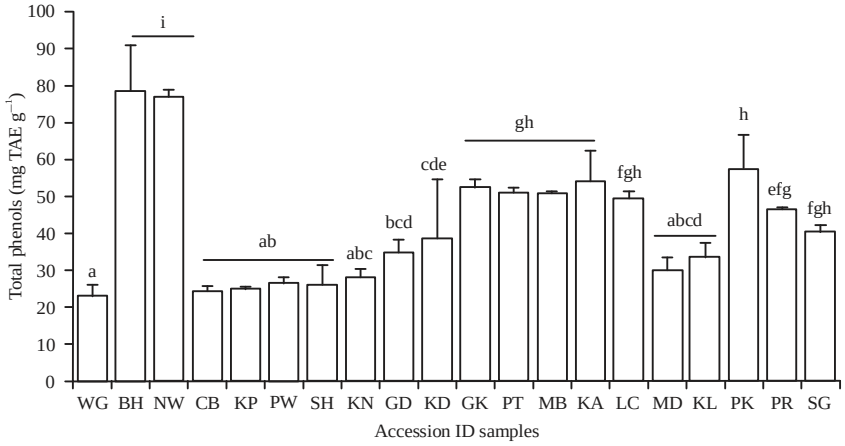

Fig. 1: Variation of total phenols contents (±SD) among different Indonesian accessions of *Curcuma aeruginosa*. Values followed by the same superscripts are not significantly different ($p<0.05$) by Duncan's test

an UV-vis spectrophotometer (Hitachi). Total phenolic content was expressed as tannic acid equivalents (mg TAE g^{-1} extract). All samples were analyzed in triplicate.

Total flavonoid contents: The total flavonoid content were determined by an aluminum chloride colorimetric assay[17] using quercetin as a reference. Stock solution. Extracts (300 mg) were mixed with 1 mL of hexamethylenetetramine (0.5%), 20 mL of acetone, 2 mL of hydrochloric acid (25%, v/v) and boiled under reflux for 30 min. The extract obtained was filtered and the residue was extracted once with 20 mL of acetone under reflux for 30 min. The extracts were combined and diluted to 100 mL with acetone. A portion of this solution, 20 mL added with 20 mL of water and extracted 3x with 15 mL of ethyl acetate. The ethyl acetate fractions were combined and diluted to 50 mL with ethyl acetate. Test solution, the stock solution (10 mL) was added with 1 mL of aluminum chloride ($AlCl_3$, 2%, w/v) and diluted to 25 mL with glacial acetic acid in methanol (5%, v/v). After 30 min, the absorbance at 425 nm was measured with an UV-vis spectrophotometer (Hitachi). Total flavonoids were expressed as quercetin equivalents (mg QE g^{-1} extract). All determinations were carried out in duplicate.

DPPH scavenging assay: The antioxidant activity of extracts were determined with DPPH scavenging assay[3] using a stable free radical 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH). Briefly, extracts (800 μg mL^{-1}) were mixed with 150 μL of ethanolic DPPH solution (300 μM) and kept in the dark for 30 min at 37°C. The absorbance at 515 nm was measured using a microplate reader (Epoch BioTek, USA). All determinations were performed in triplicate.

Statistical analysis: The data of results were expressed as Mean±Standard Deviation (SD). Analysis of variance was used to test for statistical significance difference between total phenolic and flavonoid contents and DPPH scavenging activity. Differences at $p<0.05$ confidence level were considered statistically significant. Correlation between the parameters evaluated were obtained using linear regression and Pearson correlations (0.05 significance levels). The hierarchical cluster analysis was used to group accessions based on similarities in their phenolic and flavonoid contents and DPPH scavenging activity, using SPSS 16.0 software.

RESULTS AND DISCUSSION

Variability of total phenolic and flavonoid contents of 20 *C. aeruginosa* accessions are shown in Fig. 1 and 2. Total phenolic contents ranged from 23.13±2.92 mg TAE g^{-1} for WG to 78.59±12.23 mg TAE g^{-1} for BH. Accessions BH and NW with a value 76.97±2.02 mg TAE g^{-1} showed remarkable difference with the others. These values are clearly lower than our other research[18], where the content of total phenolics was 133.0±3.7 mg TAE g^{-1}. On the other hand, total flavonoid contents of *C. aeruginosa* accessions ranged from 7.65±2.18 mg QE g^{-1} (MD) to 21.71±3.00 mg QE g^{-1} (KD). The results indicated the possibility of selecting high quality accessions for scale production or breeding programs. Several factors can be implied to explain variation total phenolic and flavonoid contents: (1) Differences in growth condition, all samples in this study were naturally grown, but in our previous study[18] cultivate were used, (2) Differences in genetic factors, there are genes that control the production of secondary metabolites in

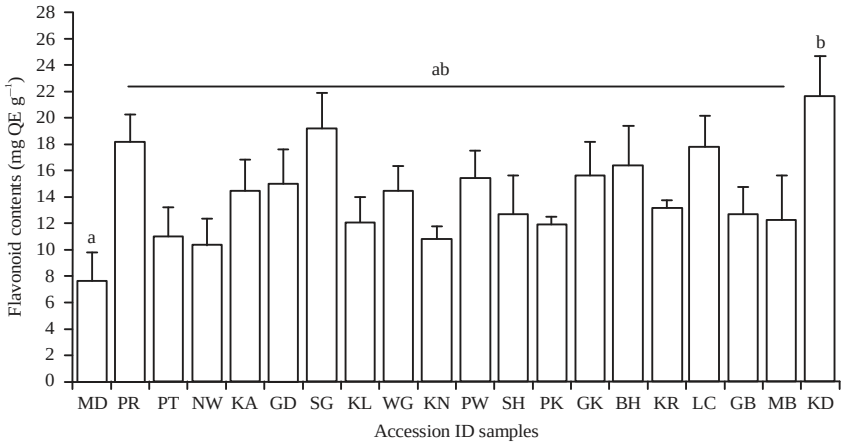

Fig. 2: Variation of flavonoid contents (±SD) in mg Quercetin Equivalent (QE) g^{-1} extracts among different Indonesian accessions of *Curcuma aeruginosa*. Values followed by the same superscripts are not significantly different ($p<0.05$) by Duncan's test

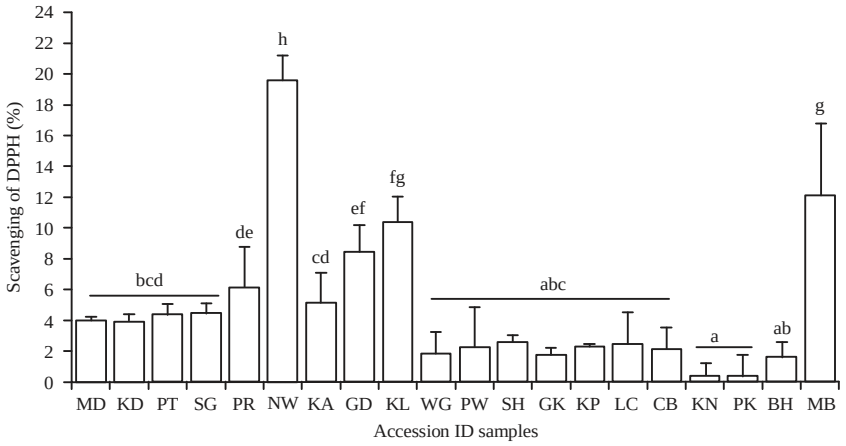

Fig. 3: Variation of DPPH radical scavenging activity (±SD) among different Indonesian accessions of *Curcuma aeruginosa*. Values followed by the same superscripts are not significantly different ($p<0.05$) by Duncan's test

plants[19] and (3) Differences in environmental conditions can effect variability in chemical composition[20].

Significant variations were found for the DPPH free radical scavenging capacity (Fig. 3) with values from 0.34±1.46 (PK) to 19.59±1.67% (NW). Phenolics and flavonoids a group of polyphenolic compounds, here known properties as free radical scavenging agents[21,22]. However, this results did not show any correlation between total phenolic and flavonoid contents and DPPH radical scavenging activity (Fig. 4). There was a low positive and negative correlation ($R^2 = 0.1514$ and Pearson correlation = 0.389 and $R^2 = 0.0475$ and Pearson correlation = -0.218) between percentage scavenging of DPPH with phenolic and flavonoid contents, respectively. This result indicated that phenolic and flavonoid in all accessions of *C. aeruginosa* are not responsible for their antioxidant activities or alternatively that radical scavenging activity (antioxidant potential) was due to specific phenol or flavonoid compounds. In other medicinal plants, the results of that study is in agreement with other reports[23].

Figure 5 presents a dendrogram of hierarchical cluster analysis to illustrate the divergence between 20 samples of *C. aeruginosa* accessions. The accessions are clustered into 3 groups based on similarities in their individual level of total phenolic and flavonoid contents and their DPPH scavenging activity. The 1st group containing 12 accessions, being comprised of CB, KP, SH, WG, PW, KN, MD, PK, PT, GD, KL and MB is characterized by result show a mix of low or high of total phenolic content (23.13-57.57 mg TAE g^{-1}) and total

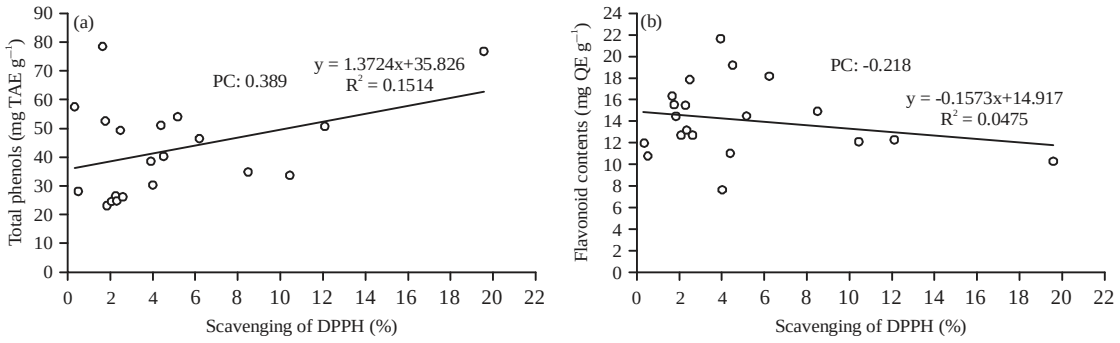

Fig. 4(a-b): Relationships between percentages scavenging of DPPH, (a) Total phenols with Pearson correlation (PC) 0.389 at the 0.05 significance levels and (b) Flavonoid contents with PC -0.218 at the 0.05 significance levels of accessions of *Curcuma aeruginosa* from Indonesia

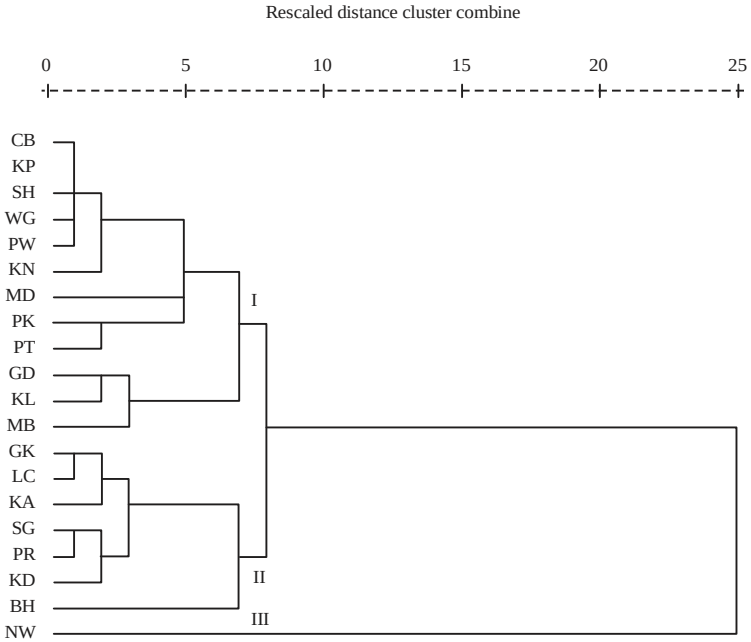

Fig. 5: Dendrogram plot visualizing the clustering of accessions of *Curcuma aeruginosa* from Indonesia based on their percentages scavenging of DPPH, total phenols and flavonoid contents

flavonoid content (7.65-15.49 mg QE g^{-1}) and low inhibition of DPPH activity (0.34-12.11%). The 2nd group comprises accession GK, LC, KA, SG, PR, KD and BH and is characterized by the highest values of total phenolic (38.64-78.59 mg TAE g^{-1}) and flavonoid contents (14.47-19.22 mg QE g^{-1}) and low DPPH scavenging activity (1.64-6.21%). The 3rd group contains only a single accession: NW and is characterized by low total flavonoid content (10.30 mg QE g^{-1}) and high of total phenolic content (76.97 mg TAE g^{-1}) and high DPPH scavenging activity (19.59%). The resulting groups of *C. aeruginosa* indicate that the accessions belong to different genetic groups. Based on their genetic diversity on metabolite of phenolics and flavonoids contents and scavenging DPPH activity, they are eligible to be used in breeding programs to produce quality and standardized materials of *C. aeruginosa*. Further investigations of the *C. aeruginosa* accessions are required to determine their genetic variation as future breeding programs through evaluations performed on individual in the same location.

CONCLUSION

Twenty accessions of *C. aeruginosa* from Indonesia showed variability in total phenolic, flavonoid contents and DPPH scavenging activity. Furthermore, evaluation performed on individual accession in the same location give a measure of genetic variability.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia with grant number 083/SP2H/PL/Dit.Litabmas/II/2015. We thank for G. John Acton, BSc. PhD for comments and English reviews that greatly improved the manuscript.

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