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Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of *Curcuma aeruginosa* accessions growing in West Java, Indonesia

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Abstract. *Khumaida N, Syukur M, Bintang M, Nurcholis W. 2019. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of* Curcuma aeruginosa *accessions growing in West Java, Indonesia. Biodiversitas 20: 656-663. Curcuma aeruginosa* is a rhizomatous medicinal plant with beneficial pharmacological activities. The aim of this work was to analyze the agro-morphological, extract yield, and phenolic content of ten *C. aeruginosa* accessions which were collected from different locations in Indonesia. Cultivation was carried out in the open field in West Java of Indonesia using a completely randomized design. Qualitative and quantitative parameters were used to investigate agro-morphological traits. Total phenolic and total flavonoids contents were determined in ethanol extracts of samples. The plants were phenotypically diverse, in which there were significant variations among the ten *C. aeruginosa* accessions in number of leaves, plant height, number of shoots, fresh weight of rhizome, and dry weight of rhizome characters. Variability in the total phenolic and total flavonoid contents ranged from 29.08-46.92 mg GAE/g, and 21.31-33.81 mg QE/g, respectively. Six accessions had high phenolic content and extract yield. Therefore, these accessions could be utilized for commercial scale and also showed a high potency for medicinal plant breeding programs.

Keywords: Accessions, agro-morphology, *Curcuma aeruginosa*, flavonoid, phenolic

INTRODUCTION

In industry, the quality of the traditional medicine product is directly related to the quality of raw materials (Salgueiro et al. 2010). However, the quality of the raw material is dependent on the breeding plant program and also the practices in the agricultural system. *Curcuma aeruginosa* Roxb., namely temu ireng or temu hitam in Indonesia, is one of the most popular rhizomatous medicinal plant belonging to the family *Zingiberaceae* and genus *Curcuma* (Sasikumar 2005). The previous works had shown that the rhizome of *C. aeruginosa* has beneficial biological activities such as antioxidant (Nurcholis et al. 2015a), antimicrobial (Kamazeri et al. 2012; Akarchariya et al. 2017), hair-growth and skin lightening (Srivilai et al. 2017), anti-androgenic (Suphrom et al. 2012), uterine relaxant (Thaina et al. 2009), and anti-dengue (Moektiwardoyo et al. 2014). In this context, *C. aeruginosa* is an important raw material for an industry of traditional medicine. Presently, there are no identified Indonesian varieties of *C. aeruginosa* (MoA 2018). Thus, the development of *C. aeruginosa* varieties is needed to produce rhizome with the highest quality for industrial purposes.

Phenolics and flavonoids have been reported as the phytochemicals found in the rhizome of *C. aeruginosa* (Nurcholis et al. 2016a). A study has demonstrated that phenolics and flavonoids possess biological activities such as antioxidant (Al-Farsi et al. 2018), antimicrobial (Pandey et al. 2018), anticancer (Alaklabi et al. 2018), anti-inflammatory and cytotoxic activity (Udavant et al. 2012). These properties make them particularly helpful for traditional medicine applications of *C. aeruginosa* rhizome. Thus, the quality of *C. aeruginosa* rhizome can be determined based on phenolics and flavonoids contents.

Research on the rhizomes of *C. aeruginosa* harvested from the different sites in Indonesia showed a high fluctuation in curcuminoid and cytotoxicity (Nurcholis et al. 2016b), phytochemical and rhizome color (Nurcholis et al. 2017), total phenolics and flavonoids contents (Nurcholis et al. 2016a). These studies suggested that the variation in bioactive content and biological activity can be influenced by several factors such as genetic and geographical variation. Also, some reports show that agro-morphological traits are controlled by a genetic factor (Belaj et al. 2011; Bakić et al. 2017). In Indonesia, there is no comprehensive study of *C. aeruginosa* accessions for agro-morphological traits, phenolics and flavonoids contents, and extractable yield. The results of this study can form a guide to facilitate a selection scheme for breeding programs for identified new cultivars of *C. aeruginosa*. Therefore, this study evaluated the morphological attributes, extract yield, and phenolic contents of ten *C. aeruginosa* accessions with grown under the same environmental conditions, so that the results reflect genetically differences between accessions studied.

MATERIALS AND METHODS

Plant material

Ten rhizomes of *C. aeruginosa* accessions were collected from different regions in Indonesia in February 2015 (Table 1). Identification of plant specimens was conducted by taxonomist expert of the Biopharmaca Conservation and Cultivation Station, Tropical Biopharmaca Research Center, Bogor Agricultural University (IPB). A field experiment was carried out at the Biopharmaca Conservation and Cultivation Station, West Java of Indonesia (6°32'25.47" N and 106°42'53.22" E, at 142.60 m altitude), in December 2015. The experiment was arranged in a completely randomized design with three-replications. The plants were grown in the same soil conditions (latosol soil with pH of 4.5-5, organic C of 1.52%, and N of 0.15%) with plants spacing of 50 cm x 50 cm. Two weeks before planting, the soil was treated with 1 kg cow manure per planting hole. Nine months after planting, in August 2016, the rhizomes were harvested.

Agro-morphological evaluations

Several qualitative and quantitative characters were measured to characterize *C. aeruginosa* accessions morphologically (Table 2). The characters were evaluated based on the set standards for traits by the Protection of Plant Varieties and Farmers' Rights Authority turmeric descriptor (PPV-FRA 2011) with modification.

Table 1. *Curcuma aeruginosa* accessions used in this work with code, collection sites, and geographical coordinates

Accession code/ Voucher specimens	Province	Location	Latitude (N)	Longitude (E)	Altitude (m)
KL/BMK0049032015	Central Java	Klewer	7˚35'05.66"	110˚49'45.38"	96
PK/BMK0053032015	Yogyakarta	Pakem	7˚39'55.46"	110˚25'11.30"	424
BH/BMK0054032015	Yogyakarta	Beringharjo	7˚47'56.40"	110˚22'01.56"	115
GK/BMK0055032015	Yogyakarta	Gunung Kidul	7˚58'04.87"	110˚36'09.67"	180
KP/BMK0056032015	Yogyakarta	Kulonprogo	7˚56'25.03"	110˚14'20.30"	20
PW/BMK0058032015	Central Java	Purworejo	7˚44'25.35"	110˚01'59.00"	56
MD/BMK0063032015	East Java	Madura	7˚02'48.90"	112˚43'47.32"	4
LC/BMK0064032015	West Java	Losari Cirebon	6˚48'17.09"	108˚48'06.04"	1
CB/BMK0065032015	West Java	Ciampea Bogor	6˚32'35.89"	106˚41'22.41"	148
MB/BMK0066032015	Jambi	Muara Bungo	1˚37'00.61"	102˚22'16.28"	65

Table 2. List of the qualitative and quantitative (agro-morphological) characters used for the variability of the 10 *C. aeruginosa* accessions

Variables	Details	Stage of observation
Qualitative variables		
Pseudostem habit (PSH)	1: compact, 9: open	150 DAP
The color on pseudostem habit (CPSH)	1: purple, 9: green	150 DAP
Venation pattern of the leaf (VPL)	3: close, 5: distant	150 DAP
Leaf disposition (LD)	3: erect (< 45°), 5: semi-erect (45°-85°), 7: horizontal (> 85°)	150 DAP
The margin of the leaf (ML)	3: even, 5: wavy	150 DAP
Purple color on midrib (PCM)	5: 50% of midrib, 7: 75% of midrib	150 DAP
Blue color on rhizome (BCR)	3: few, 5: medium, 7: many	At harvest (9 MAP)
The habit of the rhizome (HR)	3: compact, 5: intermediate, 7: loose	At harvest (9 MAP)
The shape of the rhizome (SR)	3: straight, 5: curved	At harvest (9 MAP)
Status of the tertiary rhizome (STR)	1: absent, 9: present	At harvest (9 MAP)
Length of primary rhizome (LPR)	3: short (< 5 cm), 5: medium (5 - 10 cm), 7: long (> 10 cm)	At harvest (9 MAP)
Number of mother rhizome (NMR)	1: one, 3: two-three, 5: more than three	At harvest (9 MAP)
Internode pattern of rhizome (IPR)	3: close (< 1 cm), 5: distant (> 1 cm)	At harvest (9 MAP)
Quantitative variables		
Plant height (PH)	Plant height (cm) measured from the soil level to the tip of the leaf of the main shoot	150 DAP
Pseudostem diameter (PD)	Pseudostem diameter measured in cm	150 DAP
Number of leaves (NL)	Number of leaves per plant (no.)	150 DAP
Leaf length (LL)	Leaf length in cm	150 DAP
Leaf width (LW)	Leaf width in cm	150 DAP
Number of shoots (NS)	Number of shoot per plant (no.)	150 DAP
Fresh rhizome weight (FRW)	Rhizome fresh weight per plant (kg)	At harvest (9 MAP)
Dry rhizome weight (DRW)	Rhizome dry weight per plant (kg)	At harvest (9 MAP)

Note: DAP: Days After Planting, MAP: Months After Planting

Extraction

After harvesting, the fresh rhizome of each accession was cut, dried and crushed to a powder. The powder was extracted, and performed by the maceration method of Nurcholis et al. (2015b). Briefly, 25 g of the powdered material of each accession were macerated with 70% (v/v) ethanol (250 ml) at room temperature. After 24 h, the accession solution was filtered using a Whatman filter paper (No. 4) and then subjected to evaporation (BUCHI, R-250, Switzerland) at 50°C. The extract yield of samples was calculated based on extract content (%, w/w).

Phenolic and flavonoid content

The total phenolics content of the ethanol extract was determined spectrophotometrically with the Folin-Ciocalteu method (Wan-Ibrahim et al. 2010). Extract sample (10 µL) was added to a 96-well microplate containing 160 µL distilled water. Then 10 µL of Folin-Ciocalteu reagent (10%) and 20 µL Na_2CO_3 (10%) solution were added and the mixture incubated for 30 min at room temperature. The absorbance of all accessions was measured at 750 nm using a microplate reader (Epoch BioTek, USA). All samples were analyzed in triplicate. Results of the total phenolic contents in extract samples were determined from a standard curve and expressed as mg of gallic acid equivalent per g of extract (mg GAE/g).

The total flavonoid content in ethanol extract of samples was determined spectrophotometrically using aluminum chloride reagent (Chang et al. 2002) with minor modification. The calibration curve was established using standard quercetin. Each extract or quercetin (10 µL) in a 96-well microplate was added with methanol (60 µL), 10% aluminum chloride (10 µL), 1 M potassium acetate (10 µL) and distilled water (120 µL), then the solution was incubated at room temperature for 30 min. Finally, the absorbance was determined for all samples at a wavelength of 415 nm using a microplate reader (Epoch BioTek, USA). Total flavonoid content was calculated from a standard quercetin curve and expressed as quercetin equivalents (mg QE/g).

Data analysis

The qualitative characters data were subjected to ANOVA followed by the Kruskal-Wallis H test. The data of quantitative characteristic of agro-morphologically traits, rhizome extract, total phenolics content, and total flavonoids content were subjected to ANOVA followed by Duncan's multiple range test (DMRT). Statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR) 2.0.1. All of the data were also analyzed using multivariate analysis, i.e., Bonferroni correlation, principal component analysis, and cluster analysis, performed by R software. Prior to analyses, data were normalized using log transformation and auto-scaling.

RESULTS AND DISCUSSION

Agro-morphological characters

There was no significant difference ($p < 0.05$) among ten *C. aeruginosa* accessions based on the Kruskal Wallis rank sum test of the qualitative traits (Table 3). The qualitative traits observed in the *C. aeruginosa* accessions were presented in Figure 1. The leaf disposition, blue color on the rhizome, number of mother rhizome, and internode pattern of rhizome characters showed the same pattern in all accessions. The blue color of rhizomes in this study was fewer than our previous study using samples from different geographical origin (Nurcholis et al. 2017). The accession MB has a difference for pseudostem habit, the color of the pseudostem habit, the margin of the leaf, venation pattern of the leaf, and purple color on the midrib when compared with other accessions. Based on the habit of the rhizome, the accessions BH, GK, and MD were found to be intermediate, whereas most accessions were loose. Regarding the shape of the rhizome, most accessions were straight, but the accession LC was curved. The majority of the accessions had a medium length for the primary rhizome, while the accessions KL, PW, and MD were long. Most accessions had a tertiary rhizome, but accessions MD and CB had no tertiary rhizome. The characteristics of qualitative agro-morphology were similar to the qualitative traits that were recorded by Setiadi et al. (2017). Jose and Thomas (2014) reported the presence of different morphological traits of *C. aeruginosa* including lateral spike position, purple color of the calyx, light pink color of the corolla, greenish blue color of the rhizome, dark purple color of leaf sheath, and purple-brown color of the midrib.

Table 3. Qualitative agro-morphological characters of the ten *C. aeruginosa* accessions

Accession code [a]	Qualitative agro-morphological characters [b]												
	PSH	CPSH	LD	VPL	ML	PCM	NMR	BCR	HR	SR	LPR	IPR	STR
KL	9	9	3	5	5	5	5	3	7	3	7	5	9
PK	9	9	3	5	5	5	5	3	7	3	5	5	9
BH	9	9	3	5	5	5	5	3	5	3	5	5	9
GK	9	9	3	5	5	5	5	3	5	3	5	5	9
KP	9	9	3	5	5	5	5	3	7	3	5	5	9
PW	9	9	3	5	5	5	5	3	7	3	7	5	9
MD	9	9	3	5	5	5	5	3	5	3	7	5	1
LC	9	9	3	5	5	5	5	3	7	5	5	5	9
CB	9	9	3	5	5	5	5	3	7	3	5	5	1
MB	1	1	3	3	3	7	5	3	7	3	5	5	9
H	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns	9.0ns

Note: H=value of Kruskal Wallis test; ns=nonsignificant at the 0.05 probability level; [a,b] For an explanation of accessions code and character symbols, see Table 1 and Table 2 respectively

Figure 1. Variation in the qualitative morphology of *C. aeruginosa*

The quantitative traits of the *C. aeruginosa* accessions significantly varied ($p < 0.05$) except for pseudostem diameter, leaf length, and leaf width (Table 4). The accession PW displayed highest plant height (179.14 cm), pseudostem diameter (3.51 cm), number of shoots (9.75), and fresh rhizome weight (3.90 kg/plants). The fresh and dry rhizome weight in the current study were contrastingly different from those reported by Setiadi et al. (2017) with values of 0.31 and 0.18 kg/plants, respectively. Variation in agro-morphological character is affected by environmental (Mohammadi and Asadi-Gharneh 2018), developmental (Anandan et al. 2018), and genetical (Neugart et al. 2018) factors.

Extract yield

Ethanol extract yield was varied significantly ($p < 0.05$) in different accessions (Table 5). The accession PK exhibited the highest (7.36%, w/w) ethanol extract yield, in which the accession LC was the lowest one (3.68%, w/w). The extract yield in this study was considerably lower than our previous research (7.92 to 19.71%, w/w) using the sample from a different geographic origin (Nurcholis et al. 2017). The extract yield in this study was also lower than those reported for *C. aeruginosa* by Moektiwardoyo et al. (2014) (24.13%, w/w) using the sample originated from Bandung. Extract yield is influenced by maceration time (Petropulos et al. 2014). Moektiwardoyo et al. (2014) used three days for maceration time, while in this study was two days.

Furthermore, the accessions in this research were cultivated in the same condition and extracted by technically the same method. Thus, the variation of extract yield is possibly influenced by the genetic factor in this case. The extract yield affected by genetic factor was also reported in other plant species such as *Camelina sativa* (Kurasiak-Popowska et al. 2018), *Vigna radiata* (Wang et al. 2018), *Curcuma zanthorrhiza* (Nurcholis et al. 2018), and *Curcuma zedoaria* (Syahid and Heryanto 2017).

Table 4. Quantitative agro-morphological characters of the ten *C. aeruginosa* accessions

Accession code [a]	Quantitative agro-morphological characters [b]							
	PH (cm)	**PD (cm)**	**NL (no.)**	**LL (cm)**	**LW (cm)**	**NS (no.)**	**FRW (kg/plants)**	**DRW (kg/plants)**
KL	164.99ab	3.42a	9.33ab	77.14a	17.04a	4.83cd	2.50ab	0.49ab
PK	164.39ab	3.12a	9.09ab	76.89a	15.94a	3.91d	1.80b	0.37b
BH	160.33b	3.17a	9.31ab	73.26a	15.58a	4.54d	2.10b	0.40b
GK	165.30ab	3.42a	9.67a	73.89a	16.51a	4.44d	2.13ab	0.41b
KP	172.49ab	3.30a	8.23bc	78.74a	21.37a	6.31bcd	2.90ab	0.55ab
PW	179.14a	3.51a	9.25ab	77.50a	16.89a	9.75a	3.90a	0.67ab
MD	168.99ab	3.19a	8.50abc	75.09a	15.98a	6.33bcd	3.22ab	0.60ab
LC	176.53ab	3.48a	8.75abc	77.27a	16.95a	7.75abc	3.10ab	0.75a
CB	165.49ab	2.86a	7.79c	74.54a	15.29a	5.57bcd	2.17ab	0.43b
MB	176.33ab	2.91a	7.71c	79.41a	16.14a	8.14ab	2.07b	0.38b

Note: Different letters in column indicating statistically differences mean at P < 0.05 by Duncan's multiple range test. [a,b]For an explanation of accessions code and character symbols, see Table 1 and Table 2 respectively

Phenolic and flavonoid content

Significant differences were detected among the accessions for phenolics and flavonoids contents (Table 5). The highest total phenolic content (46.92 mg GAE/g) was recorded in PK, whereas the accession KP had the lowest (29.08 mg GAE/g). Total flavonoid content ranged from 21.31 mg QE/g (MB) to 33.81 mg QE/g (GK). The previous study reported various ranges of total phenolic content (26.70 to 70.83 mg GAE/g) and total flavonoid content (7.65-21.71 mg QE/g) in the samples collected from the different geographical origin (Nurcholis et al. 2016a). Environmental factors and plant species can profoundly affect the metabolite production in medicinal plants (Oliveira et al. 2013; Moghaddam and Mehdizadeh 2015; Moghaddam and Pirbalouti 2017; Nurcholis et al. 2018). The present study was conducted in the same environment and cultivation; therefore a possible reason for total phenolic and flavonoid content variations is the genetic factor.

Table 5. Variation in ethanol extract yield, total phenolic and total flavonoid contents of ten *C. aeruginosa* accessions

Accession code [a]	Extract yield (%)	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
KL	6.01ab	32.08ab	22.14b
PK	7.36a	46.92a	23.53b
BH	6.47ab	35.08ab	26.03ab
GK	6.43ab	40.42ab	33.81a
KP	6.54ab	29.08b	23.53b
PW	5.43ab	30.42b	27.42ab
MD	6.84ab	31.08b	23.53b
LC	3.68b	37.92ab	27.42ab
CB	4.85ab	40.08ab	27.14ab
MB	6.26ab	32.58ab	21.31b

Note: Different letters in column indicate statistical differences mean at P < 0.05 by Duncan's multiple range test. For an explanation of accessions code, see Table 1

Table 6. Correlation coefficients among agro-morphology traits, extract yield, total phenolic content and total flavonoid content on ten *C. aeruginosa* accessions

	CPSH	VPL	ML	PCM	HR	SR	LPR	STR	PH	PD	NL	LL	LW	NS	FRW	DRW	Ext.	Phe.	Fla.
PSH	1.00***	1.00***	1.00***	-1.00***	-0.22	0.11	0.22	-0.17	-0.38	0.50	0.54	-0.52	0.13	-0.37	0.27	0.33	-0.089	0.19	0.41
CPSH		1.00***	1.00***	-1.00***	-0.22	0.11	0.22	-0.17	-0.38	0.50	0.54	-0.52	0.13	-0.37	0.27	0.33	-0.089	0.19	0.41
VPL			1.00***	-1.00***	-0.22	0.11	0.22	-0.17	-0.38	0.50	0.54	-0.52	0.13	-0.37	0.27	0.33	-0.089	0.19	0.41
ML				-1.00***	-0.22	0.11	0.22	-0.17	-0.38	0.50	0.54	-0.52	0.13	-0.37	0.27	0.33	-0.089	0.19	0.41
PCM					0.22	-0.11	-0.22	0.17	0.38	-0.50	-0.54	0.52	-0.13	0.37	-0.27	-0.33	0.089	-0.19	-0.41
HR						0.22	-0.048	0.22	0.49	-0.069	-0.40	0.76*	0.30	0.38	0.11	0.18	-0.38	0.0048	-0.42
SR							-0.22	0.17	0.39	0.37	-0.0057	0.15	0.037	0.30	0.27	0.65*	-0.76*	0.15	0.18
LPR								-0.22	0.18	0.41	0.27	0.068	-0.053	0.30	0.64*	0.43	0.068	-0.53	-0.23
STR									0.18	0.49	0.48	0.40	0.35	0.058	-0.084	-0.04	0.07	-0.0013	0.036
PH										0.26	-0.34	0.73*	0.34	0.94***	0.68*	0.66*	-0.45	-0.45	-0.11
PD											0.72*	0.057	0.37	0.19	0.60	0.64*	-0.22	-0.23	0.38
NL												-0.40	-0.10	-0.33	0.064	0.045	0.18	0.19	0.47
LL													0.53	0.56	0.26	0.25	-0.072	-0.36	-0.59
LW														0.16	0.34	0.31	0.065	-0.48	-0.16
NS															0.74*	0.65*	-0.49	-0.56	-0.10
FRW																0.90***	-0.37	-0.63	0.042
DRW																	-0.61	-0.42	0.088
Extract																		0.027	-0.29
Phenolic																			0.35

Note: For explanation character symbols, see Table 2. *, **, *** Significant at 0.05, 0.01, and 0.001 probability levels after Bonferroni-adjustment, respectively

Multivariate analyses

The determination of selection characters is important in plant breeding program (Acquaah 2017). Correlations between the investigated agro-morphology traits, extract yield, phenolics content of *C. aeruginosa* accessions were shown in Table 6. Results showed a positive and significant ($p \leq 0.001$) correlation between pseudostem habit (PSH), the color on pseudostem habit (CPSH), venation pattern of the leaf (VPL), and the margin of the leaf (ML); plant height (PH) and the number of shoots (NS) ($r = 0.94$). Fresh rhizome weight (FRW), which is an important characteristic in *C. aeruginosa* medicinal plant breeding of harvestable rhizomes, exhibited a significant ($p \leq 0.05$) positive correlation with NS ($r = 0.74$), PH ($r = 0.68$), and length of the primary rhizome (LPR) ($r = 0.64$). Moreover, dry rhizome weight (DRW) was significantly positively correlated with FRW ($r = 0.90$, $p \leq 0.001$), NS ($r = 0.65$, $p \leq 0.05$), pseudostem diameter (PD) ($r = 0.64$, $p \leq 0.05$), PH ($r = 0.66$, $p \leq 0.05$), and the shape of the rhizome (SR) ($r = 0.65$, $p \leq 0.05$). Leaf length (LL) was positively correlated ($p \leq 0.05$) with PH ($r = 0.73$) and the habit of the rhizome (HR) ($r = 0.76$). PD and number of leaves (NL) showed significant ($p \leq 0.05$) positive correlation ($r = 0.72$). Purple color on midrib (PCM) was significantly ($p \leq 0.001$) negative correlated with PSH, CPSH, VPL, and ML. The extract yield with SR ($r = -0.76$) and DRW ($r = -0.61$) showed a significant ($p \leq 0.05$) negatively correlation, as for total phenolic content with NS ($r = -0.56$) and FRW ($r = -0.63$) and between total flavonoid content and LL ($r = -0.59$). The improvement leading to produce a high yield extract and metabolite content such as phenolics are the main objective of plant breeding in *C. aeruginosa*. Therefore, SR, DRW, NS, and FRW can be suggested as an important selection criterion in *C. aeruginosa* for good extraction and bioactive yields. Similar result was obtained by Mishra et al., (2018) who found selection criteria such as the fresh weight of rhizome, dry weight of rhizome and plant height for improvement rhizome yield of *C. longa*.

The principal component analysis (PCA) was conducted using agro-morphology traits, extract yield, and phenolic content. The PCA allows the identification of patterns showing similarities and differences in data of the accessions studied (Kwarteng et al. 2018; Mirto et al. 2018). The first five principal components accounted for 90.041% of the cumulative contribution (total variability) (Table 7). PC-1 had 6.963 variance (Eigenvalue) which is 34.813% of total variation explained (Table 7). Purple color on leaf midrib (0.364), habit of the rhizome (0.153), status of the tertiary rhizome (0.044), plant height (0.179), leaf length (0.247), leaf width (0.003), number of shoots (0.173) and extract yield (0.023) contributed positively to PC1. In contrast, pseudostem habit (-0.364), color on pseudostem habit (-0.364), venation pattern of the leaf (-0.364), margin of the leaf (-0.364), shape of the rhizome (-0.036), length of primary rhizome (-0.075), pseudostem diameter (-0.205), number of leaves (-0.261), fresh rhizome weight (-0.072), dry rhizome weight (-0.100), phenolic (-0.093) and flavonoid (-0.217) contributed negatively to PC1. PC-2 contributed 26.139% of the total variation and demonstrated positively in the various traits such as purple color on midrib (0.051), number of leaves (0.015), extract yield (0.235), phenolic (0.262) and flavonoid (0.017). However, traits which correlated negatively to PC-2 were pseudostem habit (-0.051), color on pseudostem habit (-0.051), venation pattern of the leaf (-0.051), margin of the leaf (-0.051), habit of the rhizome (-0.173), shape of the rhizome (-0.218), length of primary rhizome (-0.193), status of the tertiary rhizome (-0.062), plant height (-0.354), pseudostem diameter (-0.264), leaf length (-0.236), leaf width (-0.231), number of shoots (-0.345), fresh rhizome weight (-0.398) and dry rhizome weight (-0.404). PC-3, PC-4, and PC5 contributed 11.181%, 9.896%, and 8.012% to the total variation, respectively.

Two-dimensional hierarchical cluster analysis (HCA) was conducted to compare with the results from PCA (Péroumal et al. 2017). The HCA allowed the relationship between the accessions studied and the studied traits to identify which of these are the most powerful (Bakić et al. 2017). Three groups were defined as shown in Figure 2. Group 1 composed only one accession (MB) with high PCM and LL traits but also low CPSH, PSH, VPL and ML traits. Group 2 was composed of three accessions: KP, PW, and LC. These accessions showed the highest DRW, FRW, NS, PH, SR and LW traits. Group 3 was comprised of MD, CB, BH, GK, KL, and PK accessions. These accessions were characterized by high total phenolic content, total flavonoid content, and extract yield. Thus, these accessions have the potential to be deployed in plant breeding programs on a commercial scale. In the second dimension, three main clusters were defined. The first group consisted of PCM, HR, LL, STR, LW, SR, PH, NS, FRW, and DRW characters. Extract yield and LPR traits were grouped in the second cluster. The third cluster contained the traits for total phenolic content, total flavonoid content, ML, VPL, PSH, CPSH, PD, and NL.

In this study, the *C. aeruginosa* accessions exhibited variations of agro-morphological traits, i.e., fresh weight of the rhizome, number of leaves, plant height, number of shoots and dry weight of rhizome. The shape of the rhizome, number of shoots, fresh rhizome weight and dry rhizome weight could be used as an important selection criterion in *C. aeruginosa* breeding for obtaining high extract and phenolics content. The accessions of MD, CB, BH, GK, KL, and PK produced high extract yield, total flavonoid, and total phenolic contents. These accessions are recommended for breeding programs resulting in high extract and the bioactive yield for commercial scale.

Table 7. Eigenvectors, Eigenvalues and proportion of variation explained by first five components for different agro-morphology traits, extract yield, total phenolic, and total flavonoid contents of *C. aeruginosa* accessions

Variable [a]	Eigenvectors				
	PC-1	PC-2	PC-3	PC-4	PC-5
PSH	-0.364	-0.051	0.014	0.026	0.180
CPSH	-0.364	-0.051	0.014	0.026	0.180
VPL	-0.364	-0.051	0.014	0.026	0.180
ML	-0.364	-0.051	0.014	0.026	0.180
PCM	0.364	0.051	-0.014	-0.026	-0.180
HR	0.153	-0.173	-0.158	-0.130	0.493
SR	-0.036	-0.218	-0.486	-0.034	-0.039
LPR	-0.075	-0.193	0.419	0.187	-0.161
STR	0.044	-0.062	-0.052	-0.662	-0.147
PH	0.179	-0.354	-0.088	0.005	-0.092
PD	-0.205	-0.264	0.048	-0.325	-0.246
NL	-0.261	0.015	0.086	-0.355	-0.347
LL	0.247	-0.236	0.053	-0.242	0.268
LW	0.003	-0.231	0.168	-0.325	0.335
NS	0.173	-0.345	-0.041	0.177	-0.165
FRW	-0.072	-0.398	0.138	0.166	-0.108
DRW	-0.100	-0.404	-0.044	0.135	-0.035
Extract yield	0.023	0.235	0.485	-0.164	0.008
Phenolic	-0.093	0.262	-0.409	-0.074	0.078
Flavonoid	-0.217	0.017	-0.292	-0.007	-0.355
Eigen value	6.963	5.228	2.236	1.979	1.602
Proportion of variation explained (%)	34.813	26.139	11.181	9.896	8.012
Cumulative proportion of variation (%)	34.813	60.952	72.133	82.029	90.041

Note: [a]For an explanation of variable symbols, see Table 2

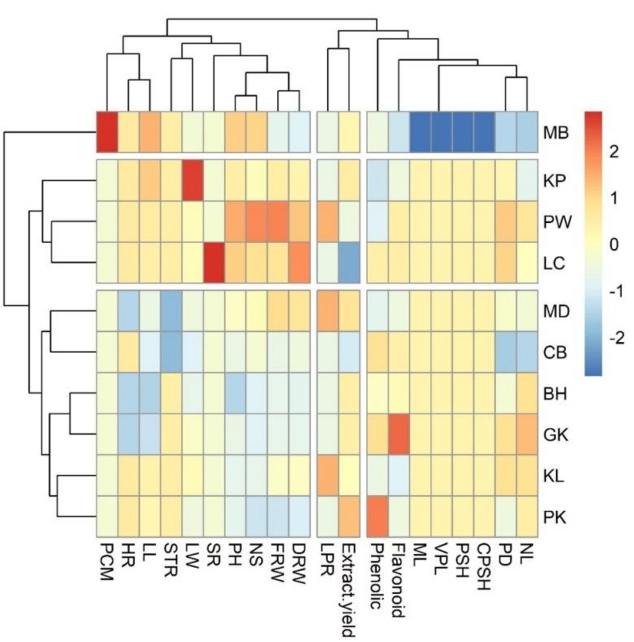

Figure 2. Heatmap and hierarchical cluster analysis of two-dimensional relationships among *C. aeruginosa* accessions and traits mainly selected for agro-morphology, extract yield, total phenolic content and total flavonoid content. For an explanation of variable symbols, see Table 2.

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