GC-MS analysis of rhizome ethanol extracts from *Curcuma aeruginosa* accessions and their efficiency activities as anticancer agent

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**Abstract.** Nurcholis W, Khumaid N, Bintang M, Syukur M. 2021. GC-MS analysis of rhizome ethanol extracts from *Curcuma aeruginosa* accessions and their efficiency activities as anticancer agent. Biodiversitas 22:1179-1186. This work aimed to evaluate the bioactive compounds and anticancer activity in rhizome extract of ten *Curcuma aeruginosa* accessions to explore their pharmacological values. The GC-MS analysis was used to identify bioactive compounds. The cytotoxicity performance was determined against MCF-7 (Human breast adenocarcinoma) and Vero cell lines using MTT assay. The GC-MS analysis revealed 71 of the compounds as sesquiterpenes (36), monoterpens (20), phenolics (5), diterpenes (4), phenanthrene (1), tetrapeptides (1), oxazole (1), triazine (1), piperidine (1), and oxygenated hydrocarbons (1). The isocurcumenol was the most dominant metabolite in ethanol extract of *C. aeruginosa* rhizome, with the highest produced by KP accession (22.01%) followed by MD accession (13.28%). Furthermore, camphor and β-elemene were among the metabolites produced by all accessions studied. In the Vero cell line as a normal cell, the cytotoxic activity varied from 13.28% (MD) to 45.17% (PW). Furthermore, the cytotoxicity activity ranged from 1.16% (LC) to 49.70% (MD) against the MCF-7 cell line. The highest anticancer activity was produced in MD accessions; thus, it can be used as a source of quality raw materials for the pharmaceutical and food industry. Besides that, it can also be further developed to obtain superior varieties through plant breeding programs.

**Keywords:** Agricultural biochemistry, bioactivities, chemometric analysis, profiling metabolite, volatile compounds

**INTRODUCTION**

*Curcuma aeruginosa*, a perennial plant belonging to the Zingiberaceae family, is used as medicinal plant. It is a native of Myanmar and distributed to Indonesia, Malaysia, Indochina, and Ceylon (Sirirugsa et al. 2007). In traditional medicine, *C. aeruginosa* is used to treat many diseases such as flatulence, dysentery, dyspepsia, and gastritis (Theanphong et al. 2015). The *C. aeruginosa* plant contains many biologically active compounds obtained from rhizomes and leaves, used as a possible medicine for various human diseases (Moektiwardoyo et al. 2014; Srivilai et al. 2018; Supphrom et al. 2012). The phytochemical compounds contained in the *C. aeruginosa* contribute to its pharmacological properties. *C. aeruginosa* contains several bioactive compounds such as flavonoids, phenolic acids (Nurcholhis et al. 2016b), curcumin, dimethoxycurcumin and bisdemethoxycurcumin (Nurcholhis et al. 2019, 2016a), germacrone (Hossain et al. 2015; Srivilai et al. 2018), isocurcumenol, zederone, cumenol, dehydrocurdione, zedoarondiol (Supphrom et al. 2012), tropolone, eucalyptol, and cumumol (Fitria et al. 2019). The reported biological activities of *C. aeruginosa* and its metabolites include antimicrobial (Akarchariya et al. 2017), analgesic effect (Reanmongkol et al. 2006), anti-androgenic (Supphrom et al. 2012), uterine relaxant effect (Thaina et al. 2009), antioxidant (Nurcholhis et al. 2017), antinociceptive (Hossain et al. 2015), hair growth promoter (Pumthong et al. 2012), and anticancer (Fitria et al. 2019). Therefore, it is crucial to select *C. aeruginosa* accessions with high phytochemical content and high pharmacological activities, which can be developed for plant breeding programs.

The composition and bioactive contents of medicinal plants can be affected by different factors, including genotypes (Batubara et al. 2020) and environmental factors (Mahajan et al. 2020; Ncube et al. 2012). The polyphenol and curcuminoids contents of *C. aeruginosa* accessions are varied by geographic location (Nurcholhis et al. 2016b, 2016a), but whether this variation is due to environmental differences or genetic variability is unclear. Recently, the GC-MS has been well established to identify different metabolites from plant extracts (Ghimire et al. 2017; Ukwubile et al. 2019), GC-MS analysis of *C. aeruginosa* essential oils identified monoterpenes and sesquiterpenes compounds associated with antibacterial activity (Akarchariya et al. 2017). Several metabolites such as terpenoids, organic acids, sterols, sugars, and fatty acids have been reported from the different extracts (methyl tert-butyl ether, methanol/chloroform) of *C. aeruginosa* using GC-MS analysis (Simoh and Zainal 2015). However, the identification of metabolites from *C. aeruginosa* accessions in ethanol extract associated with an anticancer activity using GC-MS and chemometric analyses is considerably limited.
Due to the importance of *C. aeruginosa* extract, it is necessary to investigate metabolites composition across various accessions related to pharmacological activities. Therefore, the present research focused on metabolites identification from the ethanol extract of different *C. aeruginosa* accessions and further evaluated for anticancer activity against MCF-7 cell line. We used the same soil and environment for the growth of *C. aeruginosa* rhizome; thus, the different accession results are a direct reflection of the genetic diversity. Profiling metabolites and cytotoxicity data were used to classify *C. aeruginosa* accessions using chemometric analysis. This result also showed how to choose the elite accessions to develop commercially grown varieties of *C. aeruginosa*.

**MATERIALS AND METHODS**

**Plant material and extraction**

A total of 10 *C. aeruginosa* fresh rhizome accessions obtained from various regions of Indonesia were collected in February 2015 and further identified by expert from the Tropical Biopharmaca Research Center, IPB University, Indonesia (Table 1). The rhizome of sample collection was then cultivated at the Tropical Biopharmaca Research Center in Bogor City (106°42'53.22" E, 6°32'35.89" N), West Java Province, in Indonesia at an altitude of 142.60 m and arranged with three replications using a completely randomized design. Rhizome samples were planted in the spacing of 50 cm x 50 cm and grown under the same environment for the growth of *C. aeruginosa* rhizome; organic C, and pH of 4.

All rhizome were harvested at nine months after planting. All rhizome samples were planted in the plot of 142.60 m and arranged with three replications using a completely randomized design. Rhizome samples were planted in the spacing of 50 cm x 50 cm and grown under the same environment for the growth of *C. aeruginosa* rhizome; organic C, and pH of 4.

**GC-MS analysis**

The ethanol extract of each accession was analyzed for the metabolite profile using gas chromatography-mass spectrometry (GC-MS). Previously, the sample extract (1 g) was extracted with 10 ml of hexane and then sonicated for 30 min. The filtered hexane solution (2 µl) was used for GC-MS analysis (Agilent GC 7890 series and Agilent MS 6950 series, USA) equipped with HP-5ms GC J&W capillary column (30 m x 0.25 mm i.d. and 0.25 µm film thickness). The helium was used as carrier gas with a 1 ml/min flow rate and injector temperature 250°C. The column oven temperature was programmed as follows: 40°C (hold for 2 min) to 50°C/min to 280°C as final temperature (hold for 2 min). The MS was operated at 70 eV, and the mass range scanned was 35 - 500 amu. All accessions were analyzed once without replication. The identification of compounds, including name, chemical structure, and molecular weight, was determined by adjusting the chromatogram spectra peaks with the known compounds in the NIST databases and PubChem data.

**MTT assay**

The anticancer activity was performed using breast cancer cell line MCF-7 (ATCC HTB 22) and normal Vero cells (ATCC CCL 81) as a comparison. The cytotoxic activity of sample extract was measured colorimetrically using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, St. Louis, MO, USA) according to (Vijayarathna and Sasidharan 2012) protocols with slight modification. The MTT assay is often used to measure how cells are alive and to detect the toxicity of cells. This test is attributed to the loss of a yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells. Briefly, the MCF-7 and Vero cells were cultured in Dulbecco's minimum Eagle's medium (Gibco, Rockville, MD, USA) and supplemented with fetal bovine serum (5%; Sigma-Aldrich, St. Louis, MO, USA), 100 µg/mL penicillin (Gibco, Rockville, MD, USA) and 100 µg/mL streptomycin (Gibco, Rockville, MD, USA). Cells were grown at a concentration of 5000 cells in 100 µL of growth medium. The extract (250 µg/ml) was added after the cells reached 50% confluent (24 hours). The MTT test was carried out on the third day by adding 10 µL of MTT per test well and incubating 4 hours at 37°C. Formazan crystals dissolved in ethanol. The absorbance value reading was carried out at a wavelength of 595 nm with a microplate reader (Bio-Rad 680, USA). Cytotoxicity value was calculated based on the percentage of inhibition of cell growth.

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

Table 1. The origin name and of the ten *C. aeruginosa* accessions.
Data analysis

Statistical analysis of antiproliferative data was performed by analysis of variance (ANOVA) followed by the Scott-Knott test to identify significant differences between *C. aeruginosa* accessions with R using package ‘ExpDes’. Significant differences between accessions were determined at p ≤ 0.05. R was used with package ‘heatmap’ and ‘factoextra’ for hierarchical cluster analysis (HCA)-heatmap dendrogram and principal component analysis (PCA), respectively. Graph of figure was generated using GraphPad Prism 8 for masOS (GraphPad Software Inc., San Diego, California, USA) Version 8.4.3.

RESULTS AND DISCUSSION

Metabolite compositions

Seventy-one metabolite compounds in the ethanolic extract of 10 *C. aeruginosa* accessions were successfully detected using GC-MS analysis. Those phytochemicals were identified based on retention time, peak area, and molecular formula (Table 2). From ten different extracted *C. aeruginosa* accessions the identified compounds were categorized into different groups (Figure 1) i.e sesquiterpenes (36), monoterpenes (20), phenolics (5), diterpenes (4), phenantherene (1), tetrapeptides (1), oxazole (1), triazine (1), piperidine (1), and oxygenated hydrocarbons (1). Details of each compound were presented in Table 2.

In detail, the ethanolic extract of *C. aeruginosa* accessions is mainly composed of sesquiterpenes (19.86 - 43.72%) and monoterpenes (6.3 - 25.86%). These findings correspond with research by Akarchariya et al. (2017), who reported the sesquiterpenes (45.81%) and monoterpenes (45.55%) as major components in essential oils of *C. aeruginosa* rhizome from Thailand. But, the monoterpenes were considered low than the earlier reported. The results found presented four compounds as dominant monoterpenic metabolites namely eucalyptol (3.12-9.20%), camphor (2.66-6.66%), 1, sarvone oxide (2.15-9.33%), and saussurea lactone (7.58-7.85%). Meanwhile, the dominant sesquiterpenes are β-elemene (1.16-2.67%), santonin (9.86-13.45%), herbertenolide (1.29-14.1%), epicurzerenone (2.05-12.38%), isocurcumenol (14.79-22.01%), gernacrene B (1.82-3.68%), α-cadinene (0.50-5.76%), α-guaiene (0.08-2.54%), α-farnesene (1.26-2.96%), 1,5,9-tetramethyl-2-oxatricyclo(6.4.0.0(4,8))dodecane (14.17%), 4,8-dimethyl-6-phenylazulene (1.19-2.26%), β-gurjunene (0.82-2.19%), and lactarolidole A (2.05%). The most dominant metabolite in the ethanol extract of *C. aeruginosa* rhizome is isocurcumenol, with the highest production produced by KP accession. The isocurcumenol was detected in nine accessions except for CB accession.

However, the metabolites produced by all accessions studied are camphor and β-elements, which are monoterpenes and sesquiterpenes compounds, respectively. Several researchers have reported the results of profile metabolite content in *C. aeruginosa* rhizome in different extracts based on GC-MS analysis. The study from Akarchariya et al. (2017) reported that the major components of essential oils of *C. aeruginosa* rhizome from Thailand are camphor (29.39%) and germacrone (21.21%). The work from Kamazeri et al. (2012) showed that the essential oils of *C. aeruginosa* from Malaysia contain different major compounds identified as cycloisolongifolene, 8,9-dehydro formyl (35.29%) and dihydrocostunolide (22.51%). Meanwhile, different metabolites were identified by Simoh and Zainal (2015) in various extracts, such as methenolone (16.64%), cycloisolongifolene, 8,9-dehydro-9-formyl- (15.93%), labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone (10.77%), propiolic acid, 3-(1-hydroxy)-2 isopropyl-1,5-methylcylohexylyl) (7.84%), 4-oxy-β-isodamascol (5.17%), vellerin (3.11%) and Z-α-farnesene (2.00%) in methyl tert-butyl ether extract, α-D glucopyranoside, 1,3,4,6-tetakis-O-(TMS) (trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetakis-O-(TMS)- (38.08%), d-glucose, 2,3,4,5,6-pentakis-O-(TMS)-, O-methylxime (14.61%), D-fructose, 1,3,4,5,6-pentakis-O-(TMS)-, O-methylxime (5.28%), isocitric acid (TMS) (3.06%), oxalic acid, bis (TMS) ester (2.96%), hexadecanoic acid, TMS ester (2.16%), citric acid, ethyl ester, tri-TMS (1.91%) and butanedioic acid, [(TMS) oxy]-, bis (TMS) ester (1.14%) in methanol-chloroform extract, and cycloisolongifolene, 8, 9-dehydro –9-formyl (15.70%), propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcylohexyl) (11.09%), stearic acid, TMS ester (2.78%), hexadecanoic acid, TMS ester (2.33%), oleic acid, TMS ester (1.62%), curzerene (1.56%); Z-α-farnesene (1.52%), germacrone (1.41%) and β-elemene (1.33%) in chloroform extract. Therefore, it can be shown that, based on GC-MS examination, the form of extract and the source of the raw materials decide the quantity and quality of metabolites in the *C. aeruginosa* rhizome.

In this study, a total of 5 phenolics were also identified. Xanthotoxin, coumarin 138, trimethyl (2.6 ditrr. -butyphenylox) silane, 4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene, and trans-longipinocarveol were detected. Xanthotoxin was highest detected in accession GK and MB but lowest identified in accession PK. Accession PW and MB contained coumarin 138. Meanwhile, 4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene and trans-longipinocarveol compounds were detected in accessions GK and KL, respectively. Xanthotoxin shows antidepressant (Kowalczyk et al. 2021), anticancer (Mirzaei et al. 2017; Zhang et al. 2018), anti-inflammatory (Lee et al. 2017), and anticonvulsant (Zagaja et al. 2015, 2016) activities.

Figure 1. Pie Diagram displays the percentage of compound groups found in 10 *C. aeruginosa* accessions.
Table 2. Volatile compounds identified from ethanol extract of *C. aeruginosa* rhizome accessions.

| Compounds | Group compounds | MF | MW | RT | % Total in accessions |
|-----------|-----------------|----|----|----|-----------------------|
| Xanthotoxin | Phenolics | C_{15}H_{20}O_{5} | 216 | 15.18 | 8.66 | 14.69 | 13.24 |
| Coumarin 138 | Phenolics | C_{10}H_{10}NO_{2} | 229 | 15.19 | 17.66 | 1.2 |
| 1,4,5,8,9,12-Hexahydrotriphenylene | Phenanthrene | C_{11}H_{12}O_{6} | 324 | 16.86 | 3.49 |
| Trime thyl (2,6 di tert -butyl phenox y) silane | Phenolics | C_{17}H_{10}Si | 279 | 16.99 | 1.33 |
| 6-Butyltetralin | Tetrapeptides | C_{14}H_{12} | 188 | 17.88 | 5.54 |
| 4,5-Dimethyl-1,2,3,6,7,8,8a,8b-octahydrodiphenylene | Phenolics | C_{14}H_{10} | 188 | 17.89 | 3.72 |
| Valerenol | Sesquiterpenes | C_{20}H_{26}O | 150 | 10.42 | 0.12 |
| trans-Longipinocarveol | Phenolics | C_{18}H_{20}O | 220 | 18.70 | 1.93 |
| 4-Phenylthioacetophenone | Oxazole | C_{11}H_{8}O_{2} | 228 | 19.50 | 0.41 |
| Cymetrin | Triazine | C_{8}H_{10}N_{3}S | 213 | 19.50 | 0.55 |
| 2-Piperidone | Pyrrolidine | C_{8}H_{10}NO | 99 | 19.90 | 0.61 | 0.18 |
| 2-Naonedacene | Oxygenated hydrocarbons | C_{18}H_{30}O | 282 | 20.32 | 0.36 |
| (+)-Camphene | Monoterpenes | C_{10}H_{16} | 136 | 6.29 | 0.09 |
| β-Pinene | Monoterpenes | C_{10}H_{16} | 136 | 6.69 | 0.22 | 0.08 |
| Eucalyptol | Monoterpenes | C_{10}H_{16} | 154 | 7.47 | 3.71 | 3.56 | 3.87 | 4.69 | 5.43 | 9.2 | 3.49 | 3.79 | 3.12 |
| Camphor | Monoterpenes | C_{10}H_{16} | 152 | 9.05 | 3.19 | 3.32 | 3.61 | 3.58 | 3.84 | 4.3 | 6.66 | 2.66 | 3.14 | 2.64 |
| Camphene | Monoterpenes | C_{10}H_{16} | 136 | 9.37 | 0.71 | 0.86 |
| Phenylacetaldehyde | Monoterpenes | C_{10}H_{10}O | 136 | 8.37 | 1.02 |
| α-Pinene | Monoterpenes | C_{10}H_{16} | 136 | 9.80 | 0.37 |
| Cyclopentane, 1-(1-methylithenyl)-2-(2-methyl-1-propanyl)-, (1R)-trans- | Monoterpenes | C_{10}H_{16} | 136 | 9.80 | 0.32 |
| Terpineol | Monoterpenes | C_{10}H_{16} | 154 | 9.81 | 0.5 |
| Carvone | Monoterpenes | C_{10}H_{14}O | 150 | 10.42 | 0.12 |
| 2-Carene | Monoterpenes | C_{10}H_{16} | 136 | 12.09 |
| 4-Carene | Monoterpenes | C_{10}H_{16} | 136 | 12.09 | 0.09 |
| Isoterpineol | Monoterpenes | C_{10}H_{16} | 136 | 12.10 | 0.11 |
| α-Terpine | Monoterpenes | C_{10}H_{16} | 136 | 12.12 | 0.26 |
| (+)-γ-cadinene | Monoterpenes | C_{10}H_{15} | 204 | 13.88 | 0.19 |
| β-Ionene | Monoterpenes | C_{10}H_{15} | 192 | 16.32 | 1.69 | 0.09 |
| 1-Carvone oxide | Monoterpenes | C_{10}H_{14}O | 166 | 17.17 | 7.54 | 3.34 | 9.33 | 5.45 | 2.5 | 2.37 | 2.15 |
| Saussoarea lactone | Monoterpenes | C_{10}H_{15}O | 234 | 17.17 | 7.58 | 7.85 |
| 1,3,4,4,6-Hexamethyltetralin | Monoterpenes | C_{10}H_{16} | 216 | 18.45 | 0.88 |
| 2-Isopropylidene-3-methylhexa-3,5-dienal | Monoterpenes | C_{10}H_{16} | 150 | 18.49 | 0.98 |
| δ-Elemene | Sesquiterpenes | C_{15}H_{24} | 204 | 12.11 | 0.17 |
| β-Elemene | Sesquiterpenes | C_{15}H_{24} | 204 | 12.80 | 1.51 | 1.46 | 1.75 | 1.86 | 1.72 | 2.67 | 2.36 | 1.2 | 2.14 | 1.16 |
| Caryophyllene | Sesquiterpenes | C_{15}H_{24} | 204 | 13.24 |
| (E)-β-Farnesene | Sesquiterpenes | C_{15}H_{24} | 204 | 13.50 | 0.29 |
| α-Amorphene | Sesquiterpenes | C_{15}H_{24} | 204 | 13.87 | 0.22 |
| Epizonarene | Sesquiterpenes | C_{15}H_{24} | 204 | 13.87 | 0.33 |
| Cyclo-γ-Cadinene | Sesquiterpenes | C_{15}H_{24} | 204 | 13.87 | 0.2 | 0.27 |
| Aromadendrene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.08 | 1.57 |
| Cyclosativene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.08 | 1.33 |
| Allo-Aromadendrene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.08 | 2 |
| β-Selinene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.08 | 1.6 | 1.24 | 1.52 | 1.97 | 1.27 |
| β-Guaiene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.08 | 1.21 |
| α-Selinene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.17 | 0.61 | 0.22 |
| δ-Cedrol | Sesquiterpenes | C_{15}H_{26}O | 222 | 14.35 | 0.21 |
| α-Amorphene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.35 | 0.21 |
| α-Copaene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.35 | 0.22 |
| α-Calacorene | Sesquiterpenes | C_{15}H_{24} | 200 | 14.60 | 0.15 | 0.15 |
| β-Caryophyllene | Sesquiterpenes | C_{15}H_{24} | 204 | 14.76 | 0.58 |
| Santonin | Sesquiterpenes | C_{15}H_{24}O_{5} | 246 | 15.19 | 11.21 | 13.45 | 9.86 |
| Herbertenolide | Sesquiterpenes | C_{15}H_{24}O | 230 | 15.19 | 14.1 | 1.29 |
| Epicurzerone | Sesquiterpenes | C_{15}H_{24}O | 230 | 15.20 | 12.38 | 2.24 | 3.09 | 2.42 | 2.05 | 13.4 | 7.01 | 2.18 |
| Isocurcumol | Sesquiterpenes | C_{15}H_{24}O_{2} | 234 | 15.63 | 14.79 | 15.44 | 21 | 17.08 | 22.01 | 19.72 | 21.12 | 17.49 | 5.06 |
| Germacrene B | Sesquiterpenes | C_{15}H_{24} | 204 | 15.90 | 3.68 | 1.82 |
| α-Cadinene | Sesquiterpenes | C_{15}H_{24} | 204 | 15.94 | 5.76 | 0.5 | 1.04 |
Regarding diterpenes compound, a total of 4 compounds were identified. Xeniaphyllenol B, (10-[(acetyloxy) methyl]-9-anthryl) methyl acetate, columbin, and labd-7,13-(E)-dien-15-ol were detected. The distribution between accessions of these compounds showed that the accessions GK contained xeniaphyllenol B, accession PK and MB contained (10-[(acetyloxy) methyl]-9-anthryl) methyl acetate, accession GK contained columbin, and accession BH contained labd-7,13-(E)-dien-15-ol. Columbin is known to possess anticancer (Kohno et al. 2002) and anti-inflammatory (Ibrahim Abdelwahab et al. 2012) effects.

The following six other group compounds namely phanethrene (1,4,5,8,9,12-hexahydropyrenylene), tetrapeptides (6-butyltetralin), oxazole (4-phenylthioaceto-phenone), triazine (cymetrin), piperidine (2-piperidone), and oxygenated hydrocarbons (2-nonadecanone) were also detected in accessions studied (Table 2). Accession KL contained 1,4,5,8,9,12-hexahydropyrenylene, 6-butyltetralin, and 4-phenylthioaceto-phenone compounds. Cymetrin and 2-nonadecanone were recorded in accession PK and CB, respectively. 2-Piperidone was detected in two accessions (PK and GK).

Anticancer activity

Cytotoxic activity in ethanol extract of 10 C. aeruginosa accessions against MCF-7 and Vero cell lines is presented in Figure 2. In the Vero as a normal cell, the accession PW showed maximum cytotoxic activity with a value of 45.17%. This value not significantly different from doxorubicin (45.93%), accession KP (44.89%), GK (39.74%), and KL (37.64%). In comparison, accession MD presented the lowest cytotoxic activity with a value of 15.13%. Meanwhile, in the MCF-7, the cytotoxic activity ranged between 3.04% (accession CB) to 53.65% (accession MD). All accessions studied showed significantly different from doxorubicin (65.05%) at p ≤ 0.05. A successful anticancer is that it can destroy cancer cells, but must have a limited impact on normal growth of cells (Safarzadeh et al. 2014). Results suggested selecting the accession MD to continue developing C. aeruginosa varieties with high anticancer activity through a breeding program. Atun et al. (2010) reported that methanol extract and hexane and chloroform fractions of C. aeruginosa have potent anticancer activity against the MCF-7 and Ca Ski cell lines. However, recently no study is found on ethanolic extract of C. aeruginosa for anticancer potential against the MCF-7 cell. Consequently, the results of this study have shown that the ethanolic extracts of C. aeruginosa can be produced as drug molecules for cancer disease.

Figure 2. Antiproliferative activity of the ethanol extract from the rhizome of Curcuma aeruginosa accessions against (A) Vero and (B) MCF-7 cell lines. Doxo, doxorubicin as a positive control. Values show the mean ± SD for n = 3. According to the Scott-Knott test at p ≤ 0.05, two independent studies (A and B) with different superscripts (a, b, c, d, e) differ significantly.
Multivariate analysis

In this work, the principal component analysis (PCA) and hierarchical cluster analysis (HCA)-heatmap dendrogram in *C. aeruginosa* studied accessions were used for multivariate analysis, namely chemometric. The chemometric allows for the quantification and enhancement of the understanding of metabolites information and the association of quality traits to analytical instruments data (Batubara et al. 2020). It has been used to determine the chemical components in plants that contribute to its medicinal benefits (Abbasi et al. 2018; Guo et al. 2020; Windarsih et al. 2019).

PCA analysis revealed that the first two components (shown in Figure 3) accounted for a total of 35.2% of the data variance explained. The individual score plot (Figure 3A) and loading plot (Figure 3B) represent the significant differences between the accessions studied. The diversity of data explained by PCA is low, so HCA analysis is often required to better describe the data (Khumaida et al. 2019; Péroumal et al. 2017). In chemometric analysis (PCA and HCA), it was resulted five clusters. Cluster 1 consisted of one accession viz. CB due to their high metabolite content of 4,8-dimethyl-6-phenylazulene (M62), (E)-β-farnesene (M36), δ-cedrol (M46), versalide (M67), (+)-gamma-cadinene (M27), 2-carene (M23), 2-nonenone (M12), (+)-camphene (M13), carvophyllene (M35), β-ionene (M63), and santonin (M51). Cluster 2 was composed one accession: KL. This accession presented the highest carvone (M22), 1,4,5,8,9,12-hexahydrotriphenylene (M3), isoterpinolene (M25), cyclosativene (M41), trans-longipinocarveol (M8), trimethyl(1,2,6-ditert.-butylphenoxy)silane (M4), 6-butyldihydropyrane (M5), 4-phenylthioacetophenone (M9), (+)-amorphene (M37), epicurzerenone (M53), camphene (M17), germacrene B (M55), and caryophyllene (M57) metabolites. Accession GK grouped in cluster 3. This group was characterized by high β-pinene (M14), α-selinene (M45), 4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrophenylene (M6), epizonarene (M38), terpineol (M21), Columbin (M70), xenaphyllenol B (M68), α-terpinene (M26), 1,1,3,4,4,6-hexamethyltetralin (M31), xanthotoxin (M1), and cyclo-γ-cadinene (M39). Cluster 4 consisted accession PK with characterized by high α-calacorene (M49), (10-[(acetyloxy) methyl]-9-anthryl) methyl acetate (M69), 2-piperidone (M11), cymetrin (M10), 4-carene (M24), α-amorphene (M47), valerenol (M7), and α-pinene (M19) metabolites.

Figure 3. Score plot (A), loading plot (B) and HCA-heatmap dendrogram (C) of PCA for *C. aeruginosa* accessions using the metabolites (M1 - M71, see in Table 2) and cytotoxicity activities against Vero and MCF-7 cell lines matrix as input variables. The darker red, yellow and darker blue presented higher, moderate and lower metabolites contents and cytotoxic activities, respectively.
Cluster 5 represented MB, LC, BH, MD, KP, and PW accessions. Interestingly, accessions studied in cluster 5 were associated with metabolites and cytotoxic activities. The highest cytotoxic activities against MCF-7 cell line found in accession MD (53.65%) followed with accession PW (48.91%) and KP (42.52%), but the accessions PW (45.17%) and KP (44.89%) also high cytotoxic activity against Vero cell line. These accessions had high metabolites saussurea lactone (M30), eucalyptol (M15), camphor (M16), epicurzerenone (M53), and β-elemene (M34) and cytotoxic activity against the MCF-7 cell line, indicating the association of maximum metabolites with anticancer activity. Past research has shown these compounds from several medicinal plants to be useful as anticancer. Saussurea lactone was isolated from the roots of *Saussurea lappa*, which exhibited potent anticancer properties (Robinson et al. 2008). Yang et al. (2010) showed that the eucalyptol compound from the essential oil of *Amomum tsao-ko* had anticancer activity against HepG2 carcinoma cell line. The camphor compound found in the essential oil of *Origanum vulgare* has been shown to have anti-cancer activity (Elansary et al. 2018). Rahman et al. (2013) isolated epicurzerenone (curzerenone as synonym name) from *Curcuma zedoaria*, which exhibited cytotoxicity on Ca Ski, MCF-7, and HCT-116 cancer cell lines. Meanwhile, β-elemene has been reported to have anticancer activity for several types of cancer (Deng et al. 2020; Zhai et al. 2020). The future will combine the main properties of *Curcuma aeruginosa* and *Curcuma heyneana* for the development of a new anticancer activity against cancer cells. Kowalczyk J, Nakos X, Pac J Trop Med 5:183-193. DOI: 10.1016/j.phymed.2020.153184.

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