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# Comparative Analysis of Metabolites and Antioxidant Potentials from Different Plant Parts of *Curcuma aeruginosa* Roxb.

(Analisis Bandingan Kandungan Metabolit dan Potensi Antioksidan daripada Bahagian Berbeza *Curcuma aeruginosa* Roxb.)

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ABSTRACT

*A comparative analysis of metabolites from different parts of* Curcuma aeruginosa*, i.e. leaves, stems, adventitious roots and rhizomes was performed by GC-MS/MS coupled with multivariate statistical analysis. The GC-MS/MS analysis confirmed the occurrence of 26 metabolites belonged to terpenoids in almost all the samples. The Principal Component Analysis (PCA) indicated that there was a clear distinction between rhizomes and other plant parts, i.e. stems, leaves, and adventitious roots that could be explained by relatively higher contents of terpenoids including* curzerene, alpha-farnesen, furanocoumarin, velleral, germacrone cineole, borneol, beta- *and* gamma- elemene *and* methenolone. *The results of Hierarchical Clustering Analyses (HCA) corresponded with the PCA results where many terpenoids found abundantly high in rhizome were clustered together. This was supported by the Pearson correlation analysis that showed a significantly good relationship between those terpenoids. The adventitious roots demonstrated the strongest antioxidant activity as compared to the other plant parts which could be attributed to its highest Total Phenolic Contents (TPC). Total phenolic contents of all the plant parts were positively correlated with their antioxidant activities which indicate that phenolic compounds may play a role in the overall antioxidant activities of the plants. The results of the study highlighted the potential of this underexploited* Curcuma *species which could serve as a new source of important phytochemicals and natural antioxidant that could be incorporated in functional foods and nutraceuticals. In addition, chemical and biological evidence shown in the present work has rationalised the different uses of various plant parts of* C. aeruginosa*.*

*Keywords: Antioxidant;* Curcuma aeruginosa*; GC-MS/MS; metabolomics*

ABSTRAK

*Suatu analisis perbandingan metabolit daripada bahagian berlainan* Curcuma aeruginosa, *iaitu daun, batang, akar adventisius dan rizom dijalankan dengan menggunakan GC-MS/MS berserta dengan analisis statistik multivariasi. Analisis GC-MS/MS mengesahkan kehadiran 26 metabolit dalam hampir kesemua sampel dengan kebanyakan metabolit tergolong dalam kumpulan terpenoid. Analisis Komponen Utama (PCA) menunjukkan terdapat perbezaan ketara metabolit antara rizom dan bahagian tumbuhan lain seperti batang, daun dan akar adventisius yang dapat dijelaskan dengan kandungan yang tinggi terpenoidnya, termasuk* curzerene, alfa-farnesen, furanocoumarin, velleral, germacrone cineole, borneol, beta- *dan* gama-elemene *dan* methenolone. *Keputusan analisis pengklusteran hierarki (HCA) adalah selari dengan keputusan PCA dengan sebahagian besar terpenoid yang didapati di dalam rizom dikluster bersama. Ini disokong oleh analisis korelasi Pearson yang menunjukkan perkaitan yang signifikan antara terpenoid berkenaan. Akar adventisius menunjukkan aktiviti antioksidan tertinggi berbanding dengan bahagian tumbuhan yang lain yang disebabkan oleh kandungan keseluruhan fenoliknya yang paling tinggi. Jumlah kandungan keseluruhan fenolik (TPC) daripada semua bahagian tumbuhan berkolerasi secara positif dengan aktiviti antioksidan (DPPH, FRAP) yang menunjukkan bahawa sebatian fenolik mungkin memainkan peranan penting dalam menyumbang kepada aktiviti antioksidan tumbuhan. Keputusan kajian menekankan potensi spesies* Curcuma *yang kurang dieksploitasi ini untuk menjadi satu sumber baru fitokimia dan antioksidan semula jadi yang penting yang boleh digunakan dalam makanan fungsian dan nutraseutik. Tambahan lagi, bukti kimia dan biologi yang diperoleh dalam kajian ini memberikan rasional kepada penggunaan berbeza pelbagai bahagian tumbuhan* C. aeruginosa.

*Kata kunci: Antioksida;* Curcuma aeruginosa*; GC-MS/MS; metabolomik*

## INTRODUCTION

*Curcuma aeruginosa* or locally known as Temu Hitam in Malaysia is a native plant to Southeast Asian countries including Malaysia, Thailand and Indonesia. It is one of the perennial herbs belonging to the Zingiberacea family. In Asia, a lot of studies have been conducted on *C. aeruginosa*, especially in Thailand and India.

However, in Malaysia, this herb is less known compared to other ginger plants and the potential of this herb still needs to be fully explored. The greenish-blue rhizome which emits the ginger-like aroma (Srivastava 2006) is the part mostly used in traditional medicine. However, other parts such as adventitious roots, stems and leaves are barely used in practice. The rhizome was reported to have its traditional use for gastrointestinal problems such as colic, indigestion, and stomach ache (Pandey & Chowdury 2003) and as well as a promising source of antioxidant (Choudhury et al. 2011). As far as we know, most of the previous studies carried out on this species were confined to rhizomes. Notwithstanding, a relatively better understanding on the rhizome part, the metabolite content and antioxidant potential in other parts such as adventitious roots, stems and leaves have not been well-defined. Indeed, in the Zingiberaceae family, there are different chemical properties in different parts of the plants which suggest the different uses of the species (Hans et al. 2015). The quality and level of phytochemical contents may also vary in the different plant parts (Dey et al. 2015). The analysis of metabolites using the metabolomics-based approach is crucial in order to have a holistic view of the classes of compounds present in different parts of the plant as well as to identify and profile the bioactive compounds which are associated with health beneficial properties to add value to the traditional usage of this plant. Therefore, the aims of the study were to investigate the metabolite contents in different parts of *C. aeruginosa* plants, i.e. adventitious roots, leaves, stems and rhizomes and to evaluate their antioxidant potential as well as their phenolic contents. This will provide chemical and biological evidence for the different uses of various plant parts of *C. aeruginosa*.

## Materials and Methods

### Plant materials and sample preparation

The *C. aeruginosa* Roxb. rhizomes were collected from Temerloh, Pahang (Kiza Herbs), Malaysia in April 2013. The voucher specimens (No: MDI 12804) were deposited at MARDI Herbarium, Serdang, Selangor, Malaysia. Rhizomes were germinated and cultivated under a side-netted rain shelter of 14 m long × 12 m wide × 5 m high equipped with fertigation and irrigation systems. The structures were made from galvanised steel frame and covered with 32 mesh HDPE anti-insect net. Parameters such as light intensity, humidity and temperature were depending on outside environment conditions and were not recorded. Coco peats were used as crop media to replace soil. Irrigation and fertilisation were done simultaneously and directly to the crops' adventitious roots. The leaves, stems, adventitious roots (elongated roots from the rhizomes) and rhizomes from nine-month-old fertigated plants were cut into small pieces and immediately frozen in liquid nitrogen before being ground to fine powder using mortar and pestle. The samples were then freeze-dried for a few days and kept at -20°C until further use.

### Extraction of freeze-dried *Curcuma aeruginosa* samples for GC-MS/MS analysis

The extraction procedure was performed according to the method used by Jiang et al. (2006) with a slight modification. Briefly, 10 mL methyl t-butyl ether (MTBE) (Sigma Aldrich) was added to a glass vial containing 1.0 g of freeze-dried samples of leaves, stems, adventitious roots and rhizomes. The mixtures were then incubated and shaken for 6 h at room temperature. After sonication for 10 min, the samples were centrifuged using the GSA Rotor Centrifuge at 5400 rpm for 10 min. The supernatant obtained was then filtered using a 0.2 $\mu$m PTFE filter membrane. Two hundreds microlitres of filtrate were used for GC-MS/MS analysis.

### GC-MS/MS profiling of MTBE extract of freeze-dried rhizomes

Sample volume of 1 µL was injected on a splitless mode into a GC-MS/MS system consisted of TSQ Quantum XLS GC-MS/MS (Thermo Scientific Co.). The GC column used for the analysis was TG-5MS with an inner diameter of 0.25, 30 m in length, and 0.25 µm film in thickness. Helium was used as the carrier gas at a flow rate of 1 mL $min^{-1}$. The temperature program was set according to Jiang et al. (2006) with a slight modification as following: 40°C for 2 min, then to 100°C at 7°C/min, then to 280°C at 3 C/min, then to 300°C at 6.7° C/min and hold for 3.5 min. Mass spectra were acquired using the full scan monitoring mode with a mass scan range of 50-700 m/z. The chromatogram and mass spectra were evaluated using the XCalibur software embedded in the GC-MS/MS system. The metabolite identification was performed by comparing their spectra with those contained in NIST98 database.

### Data processing and the multivariate data analysis

The GC-MS/MS data was converted to CDF form and processed through XCMS. The data was filtered and the peaks were identified. Subsequently the peaks across the samples were matched/aligned and the retention time was corrected. Finally, the missing data was filled prior to statistical analysis. The XCMS package in R version 3.0.1 was then applied to align the GC-MS/MS chromatograms with the stated values: XCMS (full width at half maximum (fwhm) = 30, step = 0.1, method = bin), group (band width = 10) (Javadi et al. 2015). The data matrix was then subjected to the multivariate data analysis with SIMCA P 11.5 software (Umetrics AB, Umea Sweden). The peak areas of the selected metabolites were compared using ANOVA test (Microsoft Excel with Data Analysis Toolpak) to confirm the significant level of metabolite differentiation among leaves, stems, adventitious roots and rhizomes of

*C. aeruginosa*. Hierarchical clustering analysis (HCA) of metabolites was performed using average metabolite values from each organ with the Pearson correlation and average linkage in MultiExperiment Viewer (MeV) software version 4.9.0 (http://www.tm4.org/mev/) (Saeed et al. 2003). Subsequently, a Pearson correlation with a two-tailed analysis was performed using average metabolite abundance values in IBM SPSS version 19 software to gain a closer look at the relationship among metabolites.

### EXTRACTION OF FREEZE-DRIED *CURCUMA AERUGINOSA* SAMPLES FOR ANTIOXIDANT ANALYSIS

Approximately 0.5 g of freeze-dried samples were weighed accurately and extracted using 20 mL of water extraction buffer containing 20 mM sodium diethyldithiocarbamic acid (Acros Organic) and 0.5% of formic acid (Fisher Scientific) (v/v). The mixture was then homogenised for 1 min, vortexed for 30 min, and finally centrifuged at 9000 rpm at 4°C for 5 min. The steps were repeated 2-6 times with 10 mL of water extraction buffer in order to obtain the sufficient amount of supernatant for antioxidant assays. The supernatant was then kept at -20°C for further use.

### DETERMINATION OF TOTAL PHENOLIC CONTENTS (TPC)

TPC of different plant parts of *C. aeruginosa* (adventitious roots, leaves, stems and rhizomes) were determined using the Folin–Ciocalteu (FC) (Merck) assay. The sample (0.3 mL) was added to 1.5 mL of 10% (v/v) FC reagent. Afterwards, 1.2 mL of 7.5% (w/v) sodium carbonate (Fisher Scientific) was added to the mixture, vortexed, and incubated for 30 min in the dark at room temperature. The absorbance was measured at 765 nm and compared to the 3-hydroxyphenyl acetic acid (Acros Organic) (as a positive control). TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried weight (mg GAE/g DW) from a calibration curve with gallic acid (Acros Organic). The values were presented as means of the triplicate analysis. Different concentrations of extracts ranging from 250 to 1000 µg/mL were used for the assay.

### DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

Antioxidant or reducing agent in *C. aeruginosa* extracts was quantified using the FRAP assay. The ferric (II) chloride (Acros Organic) solution was prepared by adding 54.1 mg of ferric (II) chloride to 10 mL of distilled water. The solution of 2,4,6-Tris (2-pyridyl)-S-triazine (TPTZ) (Fluka) was prepared by adding 31.2 mg of TPTZ to 10 mL of 40 mM HCl, whereas the acetate buffer solution was prepared by adding 0.31 g of sodium acetate in 1.6 mL of acetic acid before the volume was made up to 100 mL with distilled water and the pH was adjusted to 3.6. The working FRAP reagent was freshly prepared by mixing the ferric (II) chloride, TPTZ and acetate buffer at a ratio of 1:1:10. One and a half millilitres of the sample were then combined with 0.3 mL of distilled water before adding 3 mL of FRAP reagent. The reagent mixture was measured for the OD value at 593 nm using the spectrophotometer and compared to a 0.0625 to 4.0 mM $Fe^{2+}$. Quercetin (Acros Organic) was used as a positive control. The antioxidant capacity based on the DPPH free radical scavenging ability of the sample was expressed as mg TEAC/g dry weight (DRW) sample.

### DETERMINATION OF 1,1-DIPHENYL-2-PICRYLHYADRAZYL (DPPH) FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity *C. aeruginosa* extract was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Patel & Patel 2011). DPPH solution (40 µg/mL) was prepared by mixing 5.9 g of DPPH to 147.5 mL of methanol. DPPH solution (3 mL) was then added to 0.1 mL of sample and the mixture was incubated in the dark for 30 min. OD value was measured at 515 nm using the spectrophotometer. Ascorbic acid was used as a positive control whereas Trolox (Acros Organic) was used as a standard. The antioxidant activity based on DPPH free radical scavenging ability of the sample was expressed as mg $FeS0_4$ equivalence per 100 g DRW sample.

## RESULTS AND DISCUSSION

### METABOLOMICS ANALYSIS OF THE VARIOUS PARTS OF *CURCUMA AERUGINOSA*

The relative metabolite levels in different parts of the plants indicate the overall nutritional properties and distribution of the phytochemicals across different organs of plants of the same species (Hans et al. 2015). It is therefore necessary to comparatively profile the metabolites of the different parts of a plant for further exploitation of their health-benefiting properties. This in turn will help in identifying the most beneficial plant parts for future targeted bioactive compounds research. In the present study, the GC-MS/MS analysis of the MTBE extracts from leaves, stems, adventitious roots and rhizomes of *C. aeruginosa* resulted in the identification of 26 metabolites of which majority of them belonged to terpenoids. These included the monoterpenes such as cineol, beta- and alpha-pinene, camphor, borneol and sesquiterpenoids such as delta- and beta- elemene, curzerene and germacrone (Table 1). Saussurea lactone, alpha-tocopherol, campesterol, furanocoumarin and labd 8(20),13-diene-15-oic acid were some of the new compounds detected in the rhizomes of *C. aeruginosa* in this study, on top of previously identified compounds (Simoh & Zainal 2015). This is likely to be due to different growing conditions of sample plants used in the previous and current studies. The present study collected plants grown in the plant house with fertigation systems in which the fertiliser and irrigation were controlled whereas, in the previous study (Simoh & Zainal 2015), the plants were collected from the open field where fertiliser and irrigation systems were not known. This is taken into account that many factors such as different agro climatic condition, soil nutrition and the postharvest practices could affect the phytochemical compositions. For instance, the

study conducted by Ghasemzadeh et al. (2010) showed that the flavonoids and phenolic contents in the young rhizome of two Malaysian ginger varieties (Halia Bentong and Halia Bara) were significantly enhanced when the plants were grown under elevated $CO_2$ atmospheric concentration. Sandeep et al. (2015) reported that the concentrations of phytochemical contents including curcumin in *Curcuma longa* were very much affected by the different agro climatic zones. Similar trend of geographical influence in the variation of phytochemical contents in Zingiberacea family was also observed by Abdul Wahab et al. (2011) when they studied the essential oil composition in *C. mangga*, *C. xanthorrhiza* and *C. longa* of Malaysian varieties.

The data from GC-MS/MS analysis were then subjected to PCA analysis for better visualisation and understanding on the variation of metabolites across different parts of *C. aeruginosa*. The PCA score scatter plot generated for the first two dimensions is shown in Figure 1(a) and the loading scatter plot (Figure 1(b)) illustrated the relationship between metabolites content and respective plant parts. The principal component 1 (PC1) explained 38% of the total variance and clearly discriminated the rhizomes (located at the positive side of PC1) from the stems, leaves and adventitious roots while the principal component 2 (PC2) explained a further 25% and clearly discriminated the leaves from adventitious roots and stems (Figure 1(a)). These results indicate that the metabolites in rhizomes formed a very distinctive cluster away from the metabolites in other plant parts whilst there are some common properties shared between adventitious roots and stems of *C. aeruginosa*. The discrimination of metabolites across different plant parts could be visualised from the corresponding loading plot (Figure 1 (b)) which represents the regions of the spectra. Clearly, majority of metabolites (19 metabolites) including curzerene, furanocoumarin, velleral, germacrone cineole, borneol, beta- and gamma-elemene, methenolone and saussurea lactone were accumulated at a relatively high level in rhizomes and significantly contributed to its separation from other plant parts. Metabolites such as campesterol, alpha-tocopherol, lauric acid tetradecyl ester, and caropheyllene dominated the leaves whereas camphene and palmitic acid were abundant in adventitious roots and stems. Ghasemzadeh et al. (2016) reported that *Zingiber zerumbet*, one of the species of Zingiberace family showed the highest flavonoids content which was contributed mainly by quercetin, kaempferol and rutin in the rhizomes compared to other plant parts. Research conducted by Behar et al. (2013) also showed that a high abundance of curcuminoids in the rhizomes of two *Curcuma* species compared to the leaf part. Similar findings by Zhang et al. (2015) reported that in comparison to the aerial parts, there were significantly high amount of at least 16 different compounds observed in the rhizomes of *Alpinia officinarum*; one of the herbal plants belonged to Zingiberaceae. Based on the current findings, it is suggested that rhizome is a crucial plant part

TABLE 1. Metabolites identified in adventitious roots, leaves, stems and rhizomes of *Curcuma aeruginosa*

| | Identified metabolite | Retention times | MS fragmentation | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| 1. | Beta sitosterol | 75.19 | 107,145,213,329,414 | 414 | $C_{29}H_50$ |
| 2. | Delta Elemene | 18.64 | 91,93,121,136,161 | 204 | $C_{15}H_{24}$ |
| 3. | Beta Elemene | 20.82 | 68,81,93,107,121 | 204 | $C_{15}H_{24}$ |
| 4. | Saussurea lactone | 35.45 | 67,96,152,164,165 | 234 | $C_{15}H_{22}O_2$ |
| 5. | Beta cucubene | 8.11 | 67,69,91,93,94 | 136 | $C_{10}H_{16}$ |
| 6. | Alpha caryophyllene | 21.93 | 79, 161, 91, 93, 133 | 204 | $C_{15}H_{24}$ |
| 7. | Alpha farnesen | 23.41 | 69,93,133,161,204 | 204 | $C_{15}H_{24}$ |
| 8. | Alpha tocopherol | 71.38 | 164,165,228,430,431 | 430 | $C_{15}H_{20}O$ |
| 9. | Curzerene | 24.93 | 91,108,148,201,216 | 206 | $C_{29}H_{50}O_2$ |
| 10. | Borneol | 11.93 | 93,95,110,121,136 | 154 | $C_{10}H_{18}O$ |
| 11. | Camphene | 7.27 | 67,79,93,107,121 | 136 | $C_{10}H_{16}$ |
| 12. | Camphor | 11.54 | 69,81,95,108,152 | 152 | $C_{10}H_{16}O$ |
| 13. | Campesterol | 73.13 | 107,145,213,315,400 | 400 | $C_{28}H_{48}O$ |
| 14. | Caryophyllene | 21.89 | 79,91,93105,133 | 204 | $C_{15}H_{24}$ |
| 15. | Cholesta-22,24-dien-5-ol, 4,4-dimethyl | 73.84 | 55,83,133,207,412 | 412 | $C_{29}H_{48}O$ |
| 16. | Cineol | 8.93 | 69,71,81,108,154 | 154 | $C_{10}H_{18}O$ |
| 17. | Cycloisolongifolene-8,9-dehydro-9-formyl- | 29.07 | 94,121,162,215,230 | 230 | $C_{16}H_{22}O$ |
| 18. | Germacrone | 32.33 | 67,91,107,136,175 | 218 | $C_{15}H_{22}O$ |
| 19. | 4-oxo-alpha-isodamascol | 37.44 | 68,107,121,123,167 | 208 | $C_{13}H_{20}O_2$ |
| 20. | Furanocoumarin | 41.72 | 91,115,158,199,228 | 186 | $C_{11}H_6O_3$ |
| 21. | Labd 8(20), 13 diene-15-oic acid | 57.88 | 69,81,95,109,179 | 304 | $C_{20}H_{32}0_2$ |
| 22. | Lauric acid tetradecyl ester | 67.11 | 55,71,111,129,201 | 396 | $C_{26}H_{52}0_2$ |
| 23. | Methenolone | 53.01 | 79,81,96,123,137 | 307 | $C_{20}H_{30}O_2$ |
| 24. | Palmitic acid | 44.23 | 73,117,132,145,313 | 256 | $C_{16}H_{32}O_2$ |
| 25. | Pummerer's ketone | 34.47 | 145,159,199,214,232 | 214 | $C_{14}H_{14}O_2$ |
| 26. | Velleral | 35.75 | 108,135,147,162,232 | 232 | $C_{15}H_{20}O_2$ |

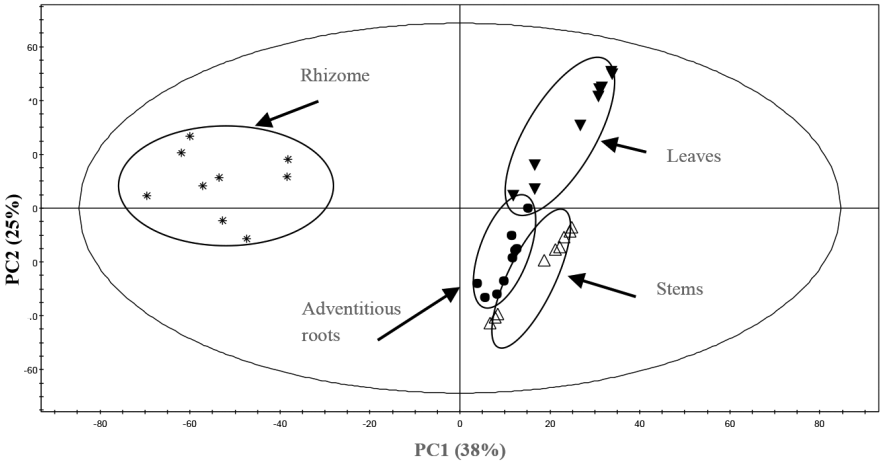


FIGURE 1(a). PCA score plot of metabolites detected from leaves, stems, adventitious roots and rhizomes of *Curcuma aeruginosa*

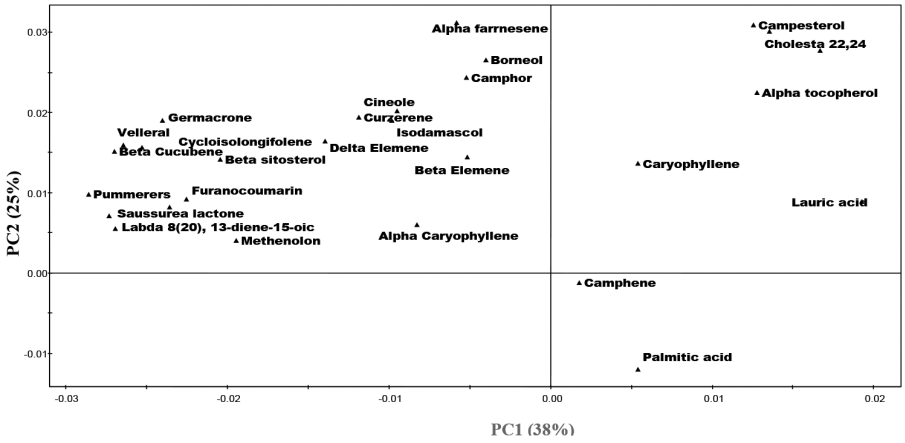


FIGURE 1(b). PCA loading plot of metabolites detected from leaves, stems, adventitious roots and rhizomes of *Curcuma aeruginosa*

in *C. aeruginosa*, where a more diverse types and higher content of secondary metabolites are readily available for medicinal purposes. The identified metabolites from rhizomes could be subjected to *in silico* prediction of ethnopharmacological properties in future study since this approach has been widely used for selected medicinal plants (Dey et al. 2015).

### HIERARCHICAL CLUSTERING AND PEARSON CORRELATION ANALYSIS OF METABOLITES IN *CURCUMA AERUGINOSA*

The relationships between the compounds available in different plant parts of *C. aeruginosa* and their abundances were investigated using HCA with a correlation heatmap (Figure 2). The HCA analysis also showed a better association between metabolites contained in adventitious root and rhizome (R=0.789) and stem and leaf extracts (R=0.75). Leaves and stems represent the aerial parts while rhizomes and adventitious roots are the underground regions of the plant. Both aerial and underground plant regions display distinct physiological and biochemical functions. The aerial parts of plant capture light energy and make photosynthates which consequently transported to rhizome and stored as reserve accumulations (e.g. starch) for overwintering and regrowth (Huang et al. 2016). The first cluster of HCA dendrogram consists of metabolites with varying abundant profile, in contrast to the second primary cluster which made up of metabolites with consistently low abundance profile across plant parts, except for alpha-tocopherol. Similarly, our PCA loading plot showed that alpha-tocopherol predominates in leaves, where it is being actively synthesised in the membrane of chloroplasts and distributed between chloroplast membranes, thylakoids and platoglobules (Mokrosnop 2014). Notably, many of the terpenoids like curzerene, germacrone, velleral, cineole, saussurea lactone and beta-elemene are clustered together, found to be enriched in the rhizome and in agreement with the results of PCA analysis. The accumulation of terpenes in rhizome may play a direct defensive role by exhibiting anti-microbial and anti-herbivore activities (Paduch et al. 2007). In addition, the release of terpenes from underground tissues

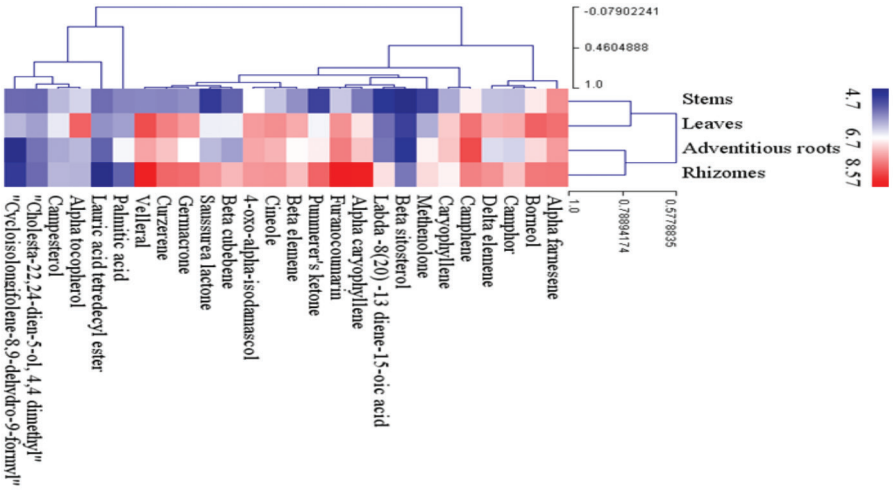


FIGURE 2. Hierarchical clustering analysis on metabolites detected from leaves, stems, adventitious roots and rhizomes of *Curcuma aeruginosa*

TABLE 2. Pearson correlation analysis between metabolites extracted from leaves, stems, adventitious roots and rhizomes of *Curcuma aeruginosa*

| | Camphene | Cineole | Camphor | Borneol | Alpha farnesene | Delta elemene | Beta elemene | Caryophyllene | Alpha caryophyllene | Curzerene | Beta cubebene | Germacrone | Punmerer's ketone | Saussurea lactone | Velleral | 4-oxo-alpha-isodamascol | Furanocoumarin | Palmitic acid | Methenolone | Labd 8(20),13 diene-15-oic acid | Cyclosiolongifolene-8,9-dehydro-9-formyl | Lauric acid tetradecyl ester | Alpha tocopherol | Campesterol | Cholesta-22,24-dien-5-ol, 4,4-dimethyl | Beta sitosterol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Camphene | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cineole | .409* | | | | | | | | | | | | | | | | | | | | | | | | | |
| Camphor | .048 | .904** | | | | | | | | | | | | | | | | | | | | | | | | |
| Borneol | .046 | .888** | .991** | | | | | | | | | | | | | | | | | | | | | | | |
| Alpha farnesene | -.203 | .672** | .818** | .799** | | | | | | | | | | | | | | | | | | | | | | |
| Delta elemene | -.099 | .412* | .442** | .474** | .402* | | | | | | | | | | | | | | | | | | | | | |
| Beta elemene | .151 | .875** | .910** | .932** | .633** | .511** | | | | | | | | | | | | | | | | | | | | |
| Caryophyllene | .766** | .468** | .269 | .324 | .029 | .262 | .424* | | | | | | | | | | | | | | | | | | | |
| Alpha caryophyllene | -.130 | .218 | .160 | .165 | .158 | .766** | .258 | -.011 | | | | | | | | | | | | | | | | | | |
| Curzerene | .023 | .371* | .370* | .430** | .293 | .720** | .520** | .513** | .599** | | | | | | | | | | | | | | | | | |
| Beta cubebene | -.142 | .468** | .398* | .392* | .315 | .610** | .500** | -.084 | .790** | .538** | | | | | | | | | | | | | | | | |
| Germacrone | -.048 | .654** | .609** | .602** | .466** | .712** | .682** | .106 | .729** | .676** | .929** | | | | | | | | | | | | | | | |
| Punmerer's ketone | -.053 | .371* | .254 | .244 | .195 | .617** | .351* | -.017 | .868** | .598** | .956** | .888** | | | | | | | | | | | | | | |
| Saussurea lactone | -.098 | .485** | .387* | .347* | .316 | .495** | .405* | -.202 | .675** | .336* | .929** | .883** | .882** | | | | | | | | | | | | | |
| Velleral | -.066 | .619** | .552** | .532** | .418* | .598** | .609** | -.028 | .722** | .529** | .968** | .970** | .917** | .951** | | | | | | | | | | | | |
| 4-oxo-alpha-isodamascol | .331* | .396* | .307 | .331* | .073 | .330* | .466** | .494** | .340* | .471** | .325 | .378* | .368* | .160 | .351* | | | | | | | | | | | |
| Furanocoumarin | -.060 | .195 | .135 | .124 | .074 | .295 | .206 | -.070 | .542** | .296 | .621** | .513** | .660** | .513** | .591** | .728** | | | | | | | | | | |
| Palmitic acid | .623** | -.084 | -.287 | -.302 | -.444** | -.296 | -.208 | .368* | -.234 | -.135 | -.381* | -.301 | -.246 | -.299 | -.323 | .144 | -.131 | | | | | | | | | |
| Methenolone | .282 | .145 | -.052 | -.067 | -.348 | .152 | .079 | .138 | .456** | .184 | .459** | .328 | .556** | .361* | .417* | .750** | .900** | .174 | | | | | | | | |
| Labd 8(20),13 diene-15-oic acid | -.058 | .462** | .342* | .301 | .289 | .444** | .357* | -.201 | .608** | .305 | .897** | .855** | .855** | .970** | .915** | .125 | .513** | -.260 | .366* | | | | | | | |
| Cyclosiolongifolene-8,9-dehydro-9-formyl | -.136 | .318 | .551** | .607** | .364* | .046 | .610** | .211 | -.300 | .106 | -.183 | -.021 | -.366* | -.307 | -.314 | .147 | -.231 | -.243 | -.376* | -.338* | | | | | | |
| Lauric acid tetradecyl ester | .017 | .197 | .379* | .375* | .367* | -.192 | .254 | .174 | -.511** | -.150 | -.528** | -.300 | -.593** | -.474** | -.377* | -.005 | -.399* | .123 | -.428** | -.516** | .635** | | | | | |
| Alpha tocopherol | .014 | .526** | .717** | .728** | .517** | .065 | .678** | .307 | -.269 | .131 | -.181 | .074 | -.316 | -.205 | -.021 | .225 | -.199 | -.120 | -.311 | -.278 | .835** | .769** | | | | |
| Campesterol | -.127 | .166 | .325 | .402* | .194 | .155 | .489** | .245 | -.107 | .205 | .009 | .034 | -.145 | -.224 | -.038 | .175 | -.099 | -.348* | -.175 | -.232 | .790** | .274 | .473** | | | |
| Cholesta-22,24-dien-5-ol, 4,4-dimethyl | .028 | .221 | .324 | .392* | .125 | .053 | .532** | .321 | -.203 | .159 | -.053 | .007 | -.213 | -.240 | -.066 | .233 | -.104 | -.160 | -.128 | -.246 | .795** | .332* | .542** | .881** | | |
| Beta sitosterol | -.070 | .298 | .245 | .241 | .214 | .665** | .368* | .147 | .782** | .741** | .773** | .794** | .838** | .643** | .729** | .286 | .404* | -.203 | .320 | .622** | -.205 | -.396* | -.158 | .046 | -.001 | |

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

is believed to initiate plant-microbe interaction with great implication on the rhizopheric microbial ecology, analogous to the roles of terpenes emitted from aerial parts such as leaves (Akiyama et al. 2005). On the other hand, Pearson correlation analysis validated that an overall good correlation among terpenes ($r$>0.418-0.970, $p$<0.05) (Table 2). For instance, velleral as one of the abundantly found terpenes shows the highest number of significant and positive correlations ($r$>0.351, $p$<0.05) with as high as 18 metabolites (69%) detected in *C. aeruginosa* (Table 2). These results could be plausibly explained by the co-expression of multiple terpene synthases responding to biotic stresses as showed by Childs et al. (2011). Terpene synthases are the key enzymes in terpene metabolism which is responsible for the conversion of precursors such as geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) into different terpene carbon skeletons (Degenhardt et al. 2009).

## THE ANTIOXIDANT CAPABILITIES OF *CURCUMA AERUGINOSA*

In this study, antioxidant activities of the various parts of *C. aeruginosa* were measured using DPPH and FRAP assays. These two different assays were performed in parallel as a combination of assays could provide more reliable results. This is due to different mechanisms of action of antioxidant activity are more accurately assessed with certain types of techniques (Frankel & Mayor 2000).

Also, the antioxidant activities of plant extracts can be easily influenced by many other factors (i.e. sample preparation, plant parts, solvent and extraction method used) (Boeing et al. 2014). On the other hand, many authors reported that the antioxidant activities are directly correlated to TPC as phenolic compounds have a strong scavenging ability to combat free radicals due to their hydroxyl groups (Khrisnaraj et al. 2010).

### TOTAL PHENOLIC CONTENTS (TPC)

Evaluation of TPC of the plant extracts from various plant parts of *C. aeruginosa* was carried out by measuring the level of phenolic compounds in the sample using Folin-Ciacalteu phenol reagent. Figure 3 shows that TPC significantly increases as the concentration of the extracts increased for most of the plant parts of *C. aeruginosa*. However, there was only a slight increment of TPC for the stems as the concentrations increased from 250 to 500 µg/mL. Adventitious roots of *C. aeruginosa* contained the highest amount of phenolics in contrast to the lowest amount observed in stems across different concentrations of individual plant extract. At the highest concentration of extract (1000 µg/mL), TPC from adventitious roots recorded the highest reading at ca. 6 g GAE/g dry weight (DRW) sample, followed by leaves (ca. 5 g GAE/ g DRW sample), rhizomes (ca. 2 g GAE/ g DRW sample) and stems (ca.1 g GAE/ g DRW sample). The same pattern was observed for the extract concentration of 500 µg/mL, where the amount of TPC in stems and rhizomes was not significantly different ($p$>0.05) from each other. At the concentration of 250 µg/mL, the amount of TPC in leaves was just slightly higher than in rhizomes as well as the amount of TPC in stems and rhizomes ($p$>0.05). In general, the ranking of TPC in different plant parts of *C. aeruginosa* in this study was in the order of adventitious roots>leaves>rhizomes>stems.

### DPPH FREE RADICAL SCAVENGING ACTIVITY

The DPPH free radical scavenging activity of various plant parts of *C. aeruginosa* is shown in Figure 4. On average, the adventitious roots of *C. aeruginosa* showed the highest DPPH scavenging activity as compared to the other plant parts. It shows that at an increased concentration of the samples, the DPPH scavenging activity of the adventitious roots also increased. The same trend was also observed in the leaves of *C aeruginosa.* At the concentration of 1000 µg/mL, the value of DPPH scavenging activity in adventitious roots exceeded 120 mg TEAC/g sample and this was almost double from the value of DPPH scavenging activity when the concentration of the sample was 500 µg/mL; i.e. circa 62 mg TEAC/g sample. For the leave sample, the DPPH scavenging activities were observed at 40 and 70 mg TEAC/g sample at the concentration of 500 and 1000µg/mL of extract, respectively. However, the DPPH scavenging activity of stems and rhizomes remained stable as the concentration of the sample increased. Figure 4 also shows that at high concentrations (i.e. 500 and 1000 µg/mL), there was a significant difference of DPPH scavenging activity between adventitious roots and rhizomes and the activities were observed to be more than double in adventitious roots at a concentration of 1000 µg/mL as compared to rhizomes. Whilst comparing between the leaves and rhizomes, only at a high concentration (1000 µg/mL), the leaves showed significantly higher DPPH scavenging activity as compared than rhizomes. At a concentration of 1000 µg/mL, the ranking of DPPH scavenging activities of different plant parts from the highest to lowest was in the order of adventitious roots>leaves>rhizomes>stems.

### FERRIC ION REDUCING ACTIVITY

In this study, the ferric reducing activity was measured using FRAP method (Benzie & Strain 1996) expressed as mg $FeS0_4$ equivalence/100 mg dry weight sample. The results (Figure 5) show that the adventitious roots of. *C. aeruginosa* gave the strongest ferric reducing activity compared to other plant parts. At the concentration of 1000 µg/mL of plant extract, adventitious roots reached the highest ferric reducing activity at ca. 1.5 mg $FeS0_4$ equivalence/100 g dry weight sample, followed by leaves (ca 1.0 mg $FeS0_4$ equivalence/100 g dry weight sample) whereas the ferric reducing activity of stems and rhizomes of *C. aeruginosa* remained at relatively lower levels in a range of 0.18 and 0.2 mg $FeS0_4$ equivalence/100 g dry weight sample. All the plant parts; adventitious roots, leaves, stems, and rhizomes of *C. aeruginosa* showed significant increments of ferric reducing

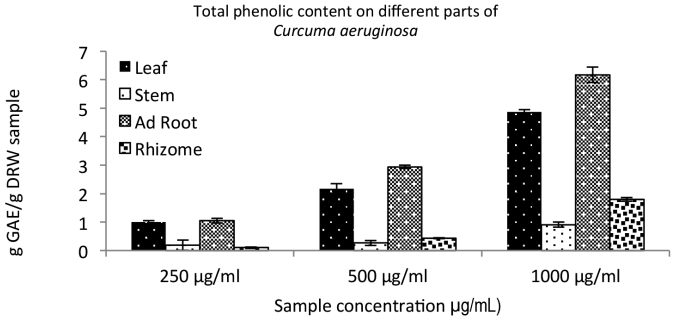


FIGURE 3. Total phenolic contents (TPC) of different plant parts of *Curcuma aeruginosa*

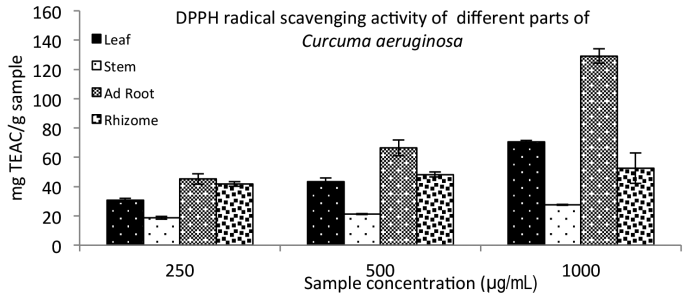


FIGURE 4. DPPH radical scavenging activity of different plant parts of *Curcuma aeruginosa*

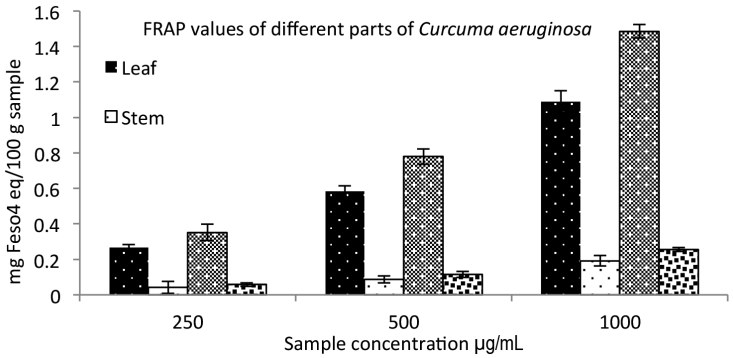


FIGURE 5. FRAP values of different plant parts of *Curcuma aeruginosa*

activity as the concentration of the plant extracts increased ($p$>0.05) except for stems and rhizomes at concentrations of 250 and 500 µg/mL. For all the concentrations, ferric reducing activities for all the plant parts were significantly different to each other ($p$<0.05) except between stems and rhizomes. In all of the concentrations (250 - 1000 µg/mL), the ranking of the ferric reducing activity of different plant parts from the highest to lowest was in the order of adventitious roots>leaves>rhizomes>stems.

The results obtained from TPC, DPPH, and FRAP assays showed that in general, the adventitious roots of *C. aeruginosa* possessed the highest phenolic content and antioxidant activity compared to the other plant parts. This was followed by the leaves, rhizomes and stems. Adventitious root is considered as non-root tissue as its formation can be induced by stress conditions (Atkinson et al. 2014). Phenolic compounds play a role in adventitious root formation as rooting co-factor (Krajnc et al. 2013). In olive, it was evident that the rooting ability of adventitious roots is affected by the oxidative enzyme activities contributed by polyphenol oxidases, an enzyme that oxidises phenolic compounds (Porfirio et al. 2016). In the present study, the highest amount of phenolic compounds present in the adventitious roots of *C. aeruginosa* in comparison to other plant parts well corresponded with the highest antioxidant activity observed. A recent study by Deepika et al. (2017) showed that phenolic compounds are significantly enhanced in the adventitious root of drought treated *Withania somnifera* with a simultaneous increase in antioxidant activity responding to oxidative injury by drought. These have been seen as a response towards stresses in adventitious roots akin to help protecting plants from oxygen reactive species (Jaleel et al. 2009). In addition, phenolics are thought to be auxin synergists due to a role of auxin-phenolic conjugates in stimulating auxin synthesis essentially for primordium initiation and development in the adventitious root (Haissig 1973). Taken together, the adventitious roots of this plant is, therefore, a potential source of beneficial secondary metabolites with antioxidant properties.

Our findings showed that both the phenolic content and antioxidant activity in the leaves of *C. aeruginosa* were the second highest, relatively greater amount of phenolic compounds compared to rhizomes and stems. These findings are in agreement with the previous report, where significantly higher total phenolic contents and antioxidant activity were found in the methanol extract of leaves of *C. aeruginosa* compared to rhizomes (Chan et al. 2008). Interestingly, these results were consistently shown in other *Curcuma* species including *C. mangga* and *C. zanthorrhiza* (Chan et al. 2008). In addition, Chan et al. (2011) observed similar outcomes from 11 species of Zingiberaceae in which their rhizomes demonstrated lower TPC than the leaves part. Ghasemzadeh et al. (2010) also reported similar findings when they analysed the TPC and antioxidant activities in leaves and rhizomes from a few ginger species.

In order to determine the relationships between TPC and antioxidant activities in different parts of *C. aeruginosa*, the

TABLE 3. Pearson correlation coefficient between TPC and antioxidant activities, DPPH and FRAP assay of different plant parts of *Curcuma aeruginosa*

| | DPPH Lf | DPPH St | DPPH Ad Rt | DPPH Rh | FRAP Lf | FRAP St | FRAP Ad Rt | FRAP Rh |
|---|---|---|---|---|---|---|---|---|
| TPC Lf | 0.992479 | - | - | - | 0.995484 | - | - | - |
| TPC St | - | 0.826993 | - | - | - | 0.700246 | - | - |
| TPC Rt | - | - | 0.996274 | - | - | - | 0.998322 | - |
| TPC Rh | - | - | - | 0.653681 | - | - | - | 0.998616 |
| DPPH Lf | - | - | - | - | 0.993526 | - | - | - |
| DPPH St | - | - | - | - | - | 0.942482 | - | - |
| DPPH Rt | - | - | - | - | - | - | 0.994451 | - |
| DPPH Rh | - | - | - | - | - | - | - | 0.658565 |

Correlation is significant at 0.05 level. Lf: Leaf, St: Stem, Ad Rt: Adventitious root, Rh: rhizome

Pearson correlation analysis was performed. Table 3 shows the positive correlations between TPC of different plant and the antioxidant activity values obtained from DPPH and FRAP assays (R=0.654- R=0.999, $p<0.05$). These results implied that phenolic compounds are likely to contribute to the overall antioxidant activities observed in all the plant parts. Our findings are in accordance with several previous reports which showed strong correlations between TPC and antioxidant activity in various plant samples (Gorinstein et al. 2004; Sellappan et al. 2002). The strong correlation between TPC and antioxidant activities has also been shown by various plant extracts of Zingiberaceae including *Curcuma* species (Angel et al. 2012; Tanvir et al. 2017).

Significant and very strong correlation coefficient values were shown between TPC and antioxidant activities by DPPH and FRAP assays in leaves and adventitious roots (R>0.99). These results imply the phenolic compounds found in those plant parts are likely to have both scavenging activity and reducing power. In contrast, TPC showed a much stronger correlation with antioxidant activity in FRAP assay (R=0.999) than in DPPH assay (R=0.654). These indicated that phenolics from rhizome extracts of *C. aeruginosa* have much higher reducing power than radical scavenging activities. Indeed, phenolic compounds act as antioxidants of which activities rely on their chemical characteristics and mechanism of action (e.g. as a metal chelator, hydrogen donators or oxygen quencher) (Galato et al. 2001). The data presented here are in accordance with the results of correlation analysis performed previously by Angel et al. (2012) which showed that TPC in the rhizomes of five *Curcuma* species were correlated stronger with reducing power than scavenging activities and *C. aeruginosa* showed the highest antioxidant activity among all the species tested.

## CONCLUSION

Our present phytochemical studies of *C. aeruginosa* showed that this plant contains a variety of biologically active compounds with antioxidant activities, which impart medicinal property. The present metabolite profiling of the leaves, stems, adventitious roots, and rhizomes of *C. aeruginosa* coupled with multivariate data analysis clearly illustrated the distinct metabolite profiles of the different parts of the plants. Our results also showed that the antioxidant activities of the adventitious roots of *C. aeruginosa* were markedly better than other plant parts examined. The findings depict the chemical and biological evidence of the plants which might be useful in determining the preferable usage of the various plant parts of *C. aeruginosa*.

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