COMPOSITION AND BIOACTIVITIES OF THE RHIZOMES ESSENTIAL OIL OF *Curcuma aeruginosa*

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Abstract

Curcuma aeruginosa is a perennial tropical herb that belongs to the Zingiberaceae family. The essential oil composition as well as antibacterial and antioxidant activities of the rhizomes of *C. aeruginosa* growing from Kluang, Johor, Malaysia were investigated. Extraction of the essential oil was conducted using the hydrodistillation technique and the chemical composition was established using gas chromatography-mass spectrometry (GC-MS). The antibacterial activity against four bacteria strains (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhi*) was determined using a disc diffusion method, while the antioxidant activity was tested using 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging method. The rhizomes of *C. aeruginosa* afforded 0.07% w/w of pure essential oil, of which 35 volatile compounds, accounting for 84.9% were successfully identified. Curzerenone (33.8%), 1,8-cineole (13.5%), and camphor (6.7%) were found as the predominant compounds in the oil. Bioactivity studies on the oil revealed that the oil demonstrated an antibacterial effect against all tested bacteria with the biggest diameter of inhibition was towards *B. cereus* (10.00 mm). However, no antioxidant activity was observed by the rhizomes essential oil of *C. aeruginosa*.

Keyword: *Curcuma aeruginosa*, essential oil, antibacterial, antioxidant, curzerenone

Introduction

Aromatic plants are a type of plant that can produce aromatic compounds, primarily essential oils. These oils not only vary in odour, flavour, density, and refractive index, but are also volatile at room temperature and have hydrophobic properties (Tongnuanchan & Benjakul, 2014; Mariod, 2016; Samarth et al., 2017). The essential oils can be derived from various plant parts such as flowers, barks, roots, leaves, buds, twigs, fruits, woods, and seeds (Burt, 2004). In nature, the plants protect themselves against pathogen attack and environmental stresses by producing essential oils (Duarte et al., 2017). Majority of the essential oils are characterised by terpene hydrocarbons (mono- and sesquiterpenes) and their oxygenated derivatives, together with aldehydes, alcohols, phenols, methoxy derivatives, and esters (Tongnuanchan & Benjakul, 2014; Elshafie & Camele, 2017). Essential oils are widely used in many industries including food and beverage, fragrances, cosmetics, aromatherapy, and household (Sharmeen et al., 2021). In addition, plant essential oils also have a broad range of medicinal uses because of their therapeutic abilities as well as numerous agro-alimentary applications due to their antimicrobial and antioxidant properties (Ríos, 2016).

Curcuma aeruginosa Roxb. (Zingiberaceae) is an aromatic perennial herb commonly recognised as temu hitam in Malaysia, pink and blue ginger in English, Waan-maa-haa-mek or kajeawdang in Thailand, and Mahamek in Hindi (Srivastava et al., 2006; Simoh & Zainal,

2015; Theanphong et al., 2015). This plant is native to Myanmar but has spread through many countries mainly in the Indochina region such as Malaysia, Indonesia, and Thailand. Apart from that, this plant can also be found in West Bengal, South Karnataka, Bihar, Coromandel coast, and Kerala (Rajkumari & Sanatombi, 2017). *C. aeruginosa* can be differentiated from other *Curcuma* species by its bluish-green rhizomes and red corolla-lobes (Sirirugsa et al., 2007). This unbranched leafy stem plant can grow up to 200 cm tall and its inflorescences are scape from the apex of the rhizomes. The *C. aeruginosa* has glabrous, alternate, and elliptic or elliptic-oblong leaves with reddish-purple leaf sheath and midrib. The colour of its bracts, coma bracts, and corollas are commonly green, pink, and yellow, respectively (Theanphong et al., 2015; Rajkumari & Sanatombi, 2017). The rhizomes of *C. aeruginosa* had long been used as traditional remedies to treat gastrointestinal problems such as diarrhoea and stomach-ache as well as other illnesses, for example, fungal infections, tumours, asthma, bronchitis, uterine pain, postpartum uterine, and perimenopausal bleeding (Simoh & Zainal, 2015; Wahyuni et al., 2017; Srivilai et al., 2018).

Investigation on the volatile compounds from the rhizomes essential oils of *C. aeruginosa* originated from various countries including Thailand, Malaysia, India, Vietnam, China, and Indonesia had been conducted by many researchers. From their studies, terpenes and terpenoids were found to be the predominant aromatic compounds in the essential oils (Oanh et al., 2018). Curzerenone (Sirat et al., 1998; Jantan et al., 1999; Sha et al., 2004; Jarikasem et al., 2005), 1,8-cineole (Jantan et al., 1999; Hartono et al., 2012; George & Britto, 2015; Wahyuni et al., 2017; Srivillai et al., 2018; Fitria et al., 2019), camphor, germacrone (Akarchariya et al., 2017), 8,9-dehydro-9-formyl-cycloisolongifolene, dihydrocostunolide (Kamazeri et al., 2012), curcumenol (Angel et al., 2014), β-pinene (Angel et al., 2014, Oanh et al., 2018), santolina triene (George & Britto, 2015), curcumanolides A,B (Zwaving & Bos, 1992), and tropolone (Fitria et al., 2019) were several major compounds identified in the essential oil. The essential oils extracted from the rhizomes of *C. aeruginosa* were reported to exhibit a wide array of bioactivities such as antibacterial (Kamazeri et al., 2012; Theanphong et al., 2015; Akarchariya et al., 2017; Wahyuni et al, 2017), antifungal (Kamazeri et al., 2012; Akarchariya et al., 2017), antinociceptive, antipyretic, anti-inflammatory (Reanmongkol et al., 2006), antimycobacterial, and antioxidant (Theanphong et al., 2015). Although many studies had been conducted on the chemical compositions and bioactivities of the rhizomes essential oil of *C. aeruginosa*, unfortunately, antioxidant and antibacterial studies of this essential oil from a specific area in Malaysia are limited. To date, only one study reported the antimicrobial properties of the rhizomes oil, in which the sample was collected from Pahang, Malaysia (Kamazeri et al., 2012). On the other hand, other studies were done by Sirat et al. (1998) and Jantan et al. (1999) only focus on the chemical composition of the rhizomes oil. Herein, we report the chemical composition, antibacterial, and antioxidant activities of the essential oil extracted from the rhizomes of *C. aeruginosa* growing in Kluang, Johor, Malaysia.

Materials and Methods

Plant material

The fresh rhizomes of *C. aeruginosa* were collected from Kluang, Johor, Malaysia in August 2019. The plant was authenticated by botanist Dr. Shamsul bin Khamis and the voucher specimen (ID008/2021) was deposited at UKMB herbarium Universiti Kebangsaan Malaysia.

Extraction of essential oil

The fresh rhizomes of *C. aeruginosa* (912.04 g) were cleaned, cut into a small size, and hydrodistilled for 8 h using a Clevenger-type apparatus. Then, the distillate was extracted with diethyl ether and dried over anhydrous magnesium sulphate. Evaporation of diethyl ether at

room temperature afforded pure essential oil and it was stored at 4ºC until further analysis (Kamazeri et al., 2012; Jani et al., 2017).

Analysis of the essential oil

The volatile compounds in the essential oil were identified using Gas chromatography-mass spectrometry (GC-MS). The analysis was accomplished with an Agilent GC-MS 7890A/5975C Series MSD (70 eV direct inlet) and equipped with HP-5MS capillary column (30 m long x 0.25 mm, 0.25 µm film thickness). The oven temperature was set from 60°C for 10 min, then gradually raised to 230°C at 3°C/min. The injector and detector temperature were programmed at 250°C and 280°C, respectively, while the helium gas was used as a carrier gas. For sample injection, 1 µL of essential oil was dissolved in diethyl ether and injected manually. The full scan mode was set to 50-550 *m/z* at 2.91 scans per second with a total scan time of 67.7 min. The volatile compounds were identified by comparing their mass spectra with those recorded in the HPCH 2205.L and NIST05a.L libraries (Jani et al., 2017).

Determination of antibacterial activity

The antibacterial activity was conducted by employing the disc diffusion method as previously described by Fadli et al. (2012) and Natta et al. (2008). The antibacterial activity was tested against two Gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 14028) as well as two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus*. The bacterial inoculum was prepared in nutrient broth and incubated at 37°C for 48 h. The inoculum bacteria were spread evenly onto the agar using a sterile cotton swab. The sterile disc (6 mm) was impregnated with 10 µL of pure essential oil. Standard antibiotic disc streptomycin (10 µg/disc) was applied as a positive control. All discs were placed onto the inoculated agar plates. Then, the plate was incubated at 37°C for 48 h. The antibacterial activity was determined by measuring the diameter of inhibition zones in millimetres and reported as mean ± standard deviation of triplicates experiments.

Determination of antioxidant activity

The DPPH assay was used in the determination of the antioxidant activity of the rhizomes essential oil of *C. aeruginosa* following the method by Tagashira & Ohtake (1998), with minor modification. One milligram of essential oil was dissolved in 1 mL of methanol to acquire the concentration of 1000 µg/mL. A freshly prepared DPPH solution (3.8 mL, 50 µM) in methanol was added to the sample solution (0.2 mL). The mixture was incubated at room temperature in the dark for 30 min. Finally, the absorbance of the reaction mixture was measured at 517 nm against methanol as a blank. A blank sample was a mixture of sample solution (0.2 mL) and methanol (3.8 mL), while blank DPPH was a mixture of methanol (0.2 mL) and DPPH solution (3.8 mL). Ascorbic acid (1000 µg/mL) was served as a positive control. The experiment was conducted in triplicates and the results were reported as mean ± standard deviation. The percentage of inhibition was computed using the following formula, where A is the absorbance:

$$\text{Percentage Inhibition (I\%)} = [(A_{\text{DPPH blank}} - [A_{\text{sample}} - A_{\text{blank sample}}]) / A_{\text{DPPH blank}}] \times 100 \quad (1)$$

Results and Discussion

Chemical composition of the essential oil

Hydrodistillation of the fresh rhizomes of *C. aeruginosa* yielded 0.07% w/w (1.37 g) pure essential oil based on the fresh weight. A total of thirty-five compounds representing 84.9% of the oil were detected. The identified compounds in the essential oil are listed in **Table 1** in order of elution on the HP-5MS capillary column. An oxygenated sesquiterpene, curzerenone

(33.8%) was the most abundant compound in the essential oil, followed by oxygenated monoterpenes, 1,8-cineole (13.5%), and camphor (6.7%). The rhizomes essential oil of *C. aeruginosa* was dominated by oxygenated sesquiterpenes (43.0%, six compounds) with curzerenone (33.8%) being the major contributor. Other oxygenated sesquiterpenes that showed a significant amount were germacrone (3.2%), curzurene (2.7%), and curcumenol (2.2%). The essential oil also consisted of eight oxygenated monoterpenes (24.4%), fourteen sesquiterpene hydrocarbons (12.6%), and seven monoterpene hydrocarbons (4.9%). To support our results, curzerenone and 1,8-cineole were also identified to be the principal compounds in the rhizomes essential oils from Malaysia (Selangor and Johor Bahru, Johor) (Sirat et al., 1998; Jantan et al., 1999) and Thailand (Jarikasem et al., 2005). However, some studies reported the absence of these compounds in their findings (Kamazeri et al., 2012; Akarchariya et al., 2017; Oanh et al., 2018; Fitria et al., 2019). The variation of chemical compounds of the essential oil of *C. aeruginosa* may be due to some factors such as geographical location, environmental factors, stage of maturity, as well as postharvest handling, and processing (Jantan et al., 2012; Oanh et al., 2018).

Table 1 Chemical composition of *C. aeruginosa* rhizomes essential oil

Rt (min)	Compound	Quality (%)	Formula	Percentage
7.30	α-Pinene	96	$C_{10}H_{16}$	0.9
8.03	Camphene	97	$C_{10}H_{16}$	1.6
9.50	Sabinene	96	$C_{10}H_{16}$	0.1
9.66	β-Pinene	94	$C_{10}H_{16}$	1.5
10.78	Myrcene	94	$C_{10}H_{16}$	0.5
13.43	1,8-Cineole	98	$C_{10}H_{18}O$	13.5
14.03	(*Z*)-β-Ocimene	97	$C_{10}H_{16}$	0.1
15.28	γ-Terpinene	97	$C_{10}H_{16}$	0.2
18.24	Linalool	90	$C_{10}H_{18}O$	0.1
20.57	Camphor	98	$C_{10}H_{16}O$	6.7
21.48	Isoborneol	95	$C_{10}H_{18}O$	1.7
22.06	Borneol	90	$C_{10}H_{18}O$	0.3
22.66	Terpinen-4-ol	98	$C_{10}H_{18}O$	0.6
23.56	α-Terpineol	91	$C_{10}H_{18}O$	1.3
26.30	Carvone	96	$C_{10}H_{14}O$	0.2
30.93	δ-Elemene	98	$C_{15}H_{24}$	0.6
33.60	β-Elemene	99	$C_{15}H_{24}$	3.5
34.77	β-Caryophyllene	99	$C_{15}H_{24}$	0.7
35.46	γ-Elemene	99	$C_{15}H_{24}$	0.1
36.57	*trans*-β-Farnesene	97	$C_{15}H_{24}$	2.6
37.38	γ-Muurolene	98	$C_{15}H_{24}$	0.1
37.59	Germacrene D	98	$C_{15}H_{24}$	1.3
37.82	β-Selinene	99	$C_{15}H_{24}$	0.8
38.20	α-Selinene	99	$C_{15}H_{24}$	0.7
38.36	Curzerene	99	$C_{15}H_{20}O$	2.7
38.64	Germacrene A	91	$C_{15}H_{24}$	0.5
39.04	γ-Cadinene	94	$C_{15}H_{24}$	0.2
39.43	δ-Cadinene	99	$C_{15}H_{24}$	0.4
40.81	Germacrene B	99	$C_{15}H_{24}$	0.9
41.96	Caryophyllene oxide	81	$C_{15}H_{24}O$	0.1
43.10	Curzerenone	90	$C_{15}H_{18}O_2$	33.8

44.47	*trans*-Muurola-3,5-diene	91	$C_{15}H_{24}$	0.2
45.01	Selin-11-en-4-α-ol	92	$C_{15}H_{26}O$	1.0
46.48	Germacrone	99	$C_{15}H_{22}O$	3.2
47.98	Curcumenol	99	$C_{15}H_{22}O_2$	2.2
	Total			**84.9**

Antibacterial activity

The essential oil was investigated for its potential to inhibit the growth of tested bacteria, *E. coli*, *S. typhi*, *B. cereus*, and *S. aureus*. The essential oil showed antibacterial activity against all tested bacteria with the diameter of inhibition zones (DIZ) ranged from 6.33 to 10.00 mm (**Table 2**). However, the inhibition zones displayed by the essential oil were lower than the positive control of streptomycin (11.00 to 20.67 mm). The highest antibacterial activity was observed against *B. cereus* and the lowest activity was demonstrated against *S. typhi*. The antibacterial activity showed by the essential oil contributed by the existence of a high quantity of oxygenated terpenes (Burt, 2004). As depicted in **Table 2**, it was found that the Gram-negative bacteria were more resistant against the *C. aeruginosa* essential oil than Gram-positive bacteria. The presence of enzymes in the periplasmic space of the Gram-negative bacteria that can break down foreign molecules as well as the existence of efflux pumps that can decrease the cellular levels of antibiotics explained the above-mentioned finding (Magina et al., 2009).

Table 2 Antibacterial activity of *C. aeruginosa* rhizomes essential oil

Bacteria strain	**Diameter of inhibition zone (mm)[a]**	
	***C. aeruginosa* oil**	**Streptomycin[b]**
Gram-negative bacteria		
E. coli	9.00 ± 0.00	11.00 ± 1.00
S. typhi	6.33 ± 0.58	15.67 ± 0.58
Gram-positive bacteria		
B. cereus	10.00 ± 1.00	20.67 ± 0.58
S. aureus	9.67 ± 0.58	18.00 ± 1.00

[a]Data represent mean ± standard deviation of three replicate experiments and include disc diameter (6 mm); [b]Standard positive control

Antioxidant activity

The DPPH assay was conducted to screen the antioxidant potential of *C. aeruginosa* rhizomes essential oil. This common, rapid, low-cost, and easy method is based on the reduction of DPPH free radical by the antioxidant, which serves as a hydrogen donor (Ahmed et al., 2018; Romanet et al., 2019). The *C. aeruginosa* essential oil was inactive towards DPPH radical since its percentage inhibition did not reach 50% of scavenging activity. The essential oil only gave 0.44 ± 0.95% of inhibition as compared to the positive control, ascorbic acid (99.15 ± 0.62%). The inactive activity was associated with the absence of phenolic compounds to transfer hydrogen atoms to the stable DPPH radical (Stanojevic et al., 2015; Theanphong et al., 2015).

Conclusion

The findings obtained from this study showed that the essential oil of rhizomes of *C. aeruginosa* originated from Kluang, Johor area was dominated by sesquiterpenes and their oxygenated derivatives (55.6%), among which curzerenone being the predominant compound. Other than that, the essential oil inhibited the growth of Gram-positive bacteria more than Gram-negative bacteria and did not scavenge DPPH radical. Since this herb has been documented to have countless medicinal uses, further bioactivity studies such as antibacterial

effect against other pathogenic bacteria, antifungal, and enzymes inhibition need to be carried out on the essential oils, extracts, and isolated phytochemicals.

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Conflict of interests

The authors declare that they have there is no conflict of interests regarding this article.

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