Bulletin of Health,
Science and Technology
BHST
ISSN 0858-7531
Volume 13, Number 1, 2015 : 06-16

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM *CURCUMA AERUGINOSA* ROXB. RHIZOME

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Abstract: This research work has been focused on the elucidation of chemical constituents and biological activities of essential oil hydrodistillated from the fresh rhizome of *Curcuma aeruginasa*. Chemical constituents of the essential oil were analyzed by GC-MS technique. Germacrone was found to be a major component (23.49%). Antioxidant activities against $DPPH^{\bullet}$, $OH^{\bullet}$, $H_2O_2^{\bullet}$ radicals and reducing power of the essential oil were evaluated. As compared to *L*-ascorbic acid as a positive control, it was found that there was no significantly statistical difference in the scavenging effectiveness toward $DPPH^{\bullet}$ and $OH^{\bullet}$ radicals between the essential oil (EC_{50} = 24.32 and 244.48 μg/ml, respectively) and *L*-ascorbic acid (EC_{50} = 21.21 and 228.24 μg/ml, respectively) while the essential oil was statistically significant less effective than *L*-ascorbic acid in scavenging effectiveness toward $H_2O_2^{\bullet}$. For reducing power assay, the essential oil showed no activity. Results of antibacterial activity testing by agar diffusion technique showed that the essential oil has modulate activity against *Enterococcus faecalis* ATCC 29212 with MIC value of 6.25 μg/ml. Tetracyclin, the positive control, exhibited stronger activity against all bacteria tested with MIC < 0.04 μg/ml. Antimycrobacterial activity of the essential oil examined by green fluorescent protein microplate assay (GFPMA) was weaker activity against *Mycobacterium tuberculosis* H37Ra (MIC value of 2500 mg/ml). Isoniazid, the positive control, gave the MIC value of 0.023-0.046 μg/ml.

Keywords: *Curcuma aeruginosa*, Essential oil, Antioxidant, Antibacterial, Antituberculosis

บทคัดย่อ: งานวิจัยนี้เป็นการศึกษาองค์ประกอบทางเคมี และฤทธิ์ทางชีวภาพ ของน้ำมันระเหยง่ายจากเหง้าสดของว่านมหาเมฆ ที่ได้จากการกลั่นด้วยน้ำ จากการศึกษาองค์ประกอบทางเคมีของน้ำมันระเหยง่ายโดยเทคนิค GC-MS พบว่า germacrone (23.49%) เป็นองค์ประกอบทางเคมีหลัก จากการทดสอบฤทธิ์ต้านอนุมูลอิสระ $DPPH^{\bullet}$ $OH^{\bullet}$, $H_2O_2^{\bullet}$ และ reducing power ของน้ำมันระเหย พบว่า น้ำมันระเหยง่ายและ *L*- ascorbic acid ซึ่งเป็นสารมาตรฐาน มีความสามารถ ในการต้านอนุมูลอิสระ $DPPH^{\cdot}$ และ $OH^{\cdot}$ ใกล้เคียงกัน โดยค่า EC_{50} ของน้ำมันระเหยง่าย มีค่าเท่ากับ 24.32 และ 244.48 μg/ml ตามลำดับ ในขณะที่ค่า EC_{50} ของ *L*-ascorbic acid มีค่าเท่ากับ 1.21 และ 228.24 μg/ml ตามลำดับ นอกจากนี้ยังพบว่าน้ำมันระเหยง่ายมีฤทธิ์ต้านอนุมูลอิสระ $H_2O_2^{\bullet}$ ต่ำกว่า *L*-ascorbic acidอย่างมีนัยสำคัญ การศึกษา reducing power assay พบว่าน้ำมันระเหยง่ายไม่มีฤทธิ์ดังกล่าว ผลการทดสอบฤทธิ์ต้านแบคทีเรียโดยวิธี agar diffusion พบว่า น้ำมันระเหยง่ายมีฤทธิ์ปานกลางในการยับยั้งเชื้อ *Enterococcus faecalis* ATCC 29212 โดยมีค่า MIC = 6.25 μg/ml ในขณะที่ tetracyclin ซึ่งเป็นสารมาตรฐานมีฤทธิ์ต้านเชื้อแบคทีเรียทุกชนิดที่ใช้ทดสอบดีกว่าน้ำมันระเหยง่าย โดยมีค่า MIC น้อยกว่า 0.04 μg/ml จากการทดสอบฤทธิ์ต้านเชื้อวัณโรค (*Mycobacterium tuberculosis* H37Ra) โดยวิธี green fluorescent protein microplate assay (GFPMA) พบว่า น้ำมันระเหยง่ายมีฤทธิ์ต้านเชื้อวัณโรคต่ำกว่า isoniazid ซึ่งเป็นสารมาตรฐาน (MIC ของน้ำมันระเหยง่ายและสารมาตรฐานมีค่าเท่ากับ 2500 mg/ml และ 0.023-0.046 μg/ml ตามลำดับ)

คำสำคัญ: ว่านมหาเมฆ น้ำมันระเหยง่าย ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านแบคทีเรีย ฤทธิ์ต้านวัณโรค

INTRODUCTION

Essential oils are a group of compounds generally found in aromatic plants. Several essential oils exhibit interesting biological activity such as antimicrobial (Singh *et al*., 2008; Sahebkar and Iranshahi, 2010; Angel, Vimala and Bala, 2012; Saha *et al*., 2013), antiviral (Adorjan and Buchbauer, 2010), antifungal (Jantan *et al*., 2003) anti-inflammatory (Vendruscolo *et al*., 2006) antituberculosis (Başer *et al*., 2009; Bueno-Sánchez *et al*., 2009; Sergio *et al*., 2013) and antioxidant (Bua-in and Paisooksantivatana, 2009; Al-Reza *et al*., 2010; Liju *et al*., 2011; Tsai *et al*., 2011) activities.

Plants in the family Zingiberaceae are considered as important sources of food, spices, medicines, dyes, perfumes and cosmetics. Many of them are also cultivated for their economic uses or as ornamental plants. Several species have been used ethnomedically in various countries.

Curcuma aeruginosa Roxb., which belongs to the family Zingiberaceae, is an aromatic perennial herb with 30-40 cm in height. Its leaves are glabrous, alternate, elliptic or elliptic-oblong, entire, reddish-purple leaf sheath and midrib. The rhizome is bullish violet, ellipsoid-ovate shape. The inflorescences are scape from the apex of the rhizome. Bracts are green. Coma bracts are pink. Corolla is yellow. The ethnomedical uses of this plant are treatment of dysentery, gastritis, dyspepsia and flatulence (Neamsuvan *et al*., 2012; Nanda *et al*., 2013). To the best of our knowledge, there are very little information available on chemical compositions and biological activities of the essential oil from *C. aeruginosa* fresh rhizome. In the present study chemical compositions of the essential oil from the fresh rhizome of *C. aeruginosa* was identified and evaluated for antioxidant, antibacterial and antimycrobacterial activities. Effective concentration (EC_{50}) and minimum inhibitory concentration (MIC) of the essential oil were also evaluated.

MATERIALS AND METHODS

Plant material

The whole plants of *C. aeruginosa* were collected from Ratchaburi province, Thailand. The plant sample was identified by comparison with herbarium specimens at the Singapore Herbarium (Singapore Botanic Gardens, Singapore) voucher no. SING 0042078. The voucher specimen of this plant was deposited at Faculty of Pharmacy, Rangsit University, Patumtani, Thailand.

Isolation of essential oil

The fresh rhizomes of *C. aeruginosa* (300 g) were washed with tap water, air dried and then blended into small pieces with the blender. The ground rhizome of the plant sample was subjected to water distillation using Clevenger apparatus for 3 hr. The essential oil was collected and stored at 4°C in air-tight containers before analyzed by GC-MS technique.

GC-MS Analysis

Essential oil was diluted with the oil to ethanol ratio of 1:100 by volume (HPLC grade, RCI Labscan, Thailand) and analyzed by a Finnigan Trace GC ultra-equipped with a Finnigan DSQ quadrupole mass spectrometric detector (MSD). The BPX5 (Phenyl: Dimethylpolysiloxane 5:95) capillary column (30 m in length, 0.25 mm i.d., and 0.25 μm in thickness) was used as stationary phase. The carrier gas was Helium with the flow rate of 1 ml/min. 1μl of diluted oil was injected using Finnigan Autosampler AI 3000 (split ratio 1:100). The operating condition of GC oven temperature was started at 60°C, held it for

1 min, ramped at the rate of 3°C/min to 240°C and held for 5 min. The GC injector and GC-MSD interface temperatures were set at 180°C and 290°C, respectively. MS operating parameters were followed: ion source 200°C; electron impact ionization positive mode at 70 eV with scaning mass range of 40-650 m/z, scaning rate 500 amu / second.

Identification of oil components

Essential oil components were identified by comparing their mass fragmentation pattern with Adams Essential Oil Mass Spectral library and NIST05 Mass Spectral library. The amount of each oil component was determined on the basis of peak area measurement.

Antioxidant activity

DPPH scavenging assay

The DPPH free-radical scavenging activity of the essential oil was determined according to the method reported by Wang, *et al.* (2008). 1 ml of different concentrations of the essential oil in methanol (0-5000 µg/ml) was mixed with 2 ml of 6×10^{-5} M DPPH solution in methanol. The reaction mixture was well shaken and kept it in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517 nm against the blank, i.e. methanol. *L*-ascorbic acid was used as a positive control. The percentage of scavenging DPPH radicals was calculated as follows:

$$\text{Percent inhibition} = [1-(A_1-A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control (without the essential oil), A_1 is the absorbance of the reaction mixture and A_2 is the absorbance of the sample without DPPH.

The EC_{50} value, the effective concentration at which the DPPH scavenging effect being 50% was obtained by linear equation from graph plotted between scavenging percentage and essential oil concentration.

$OH^{\bullet}$ scavenging assay

$OH^{\bullet}$ scavenging assay was performed according to the method of Wang, *et al*. (2008) with some modifications. 1 ml of different concentrations of the essential oil in methanol (0-5000 µg/ml) was mixed with a mixture of 1.5 ml of 1.5 mM FeSO4, 0.7 ml of 6 mM H_2O_2 and 0.3 ml of 20 mM sodium salicylate. The reaction mixture was incubated at 37°C for 1 hr. The absorbance of the reaction mixture was measured at 562 nm against the blank, i.e. methanol. *L*-ascorbic acid was used as a positive control. The percentage of scavenging $OH^{\bullet}$ radicals was calculated as follows:

$$\text{Percent inhibition} = [1-(A_1-A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control (without the essential oil), A_1 is the absorbance of the reaction mixture and A_2 is the absorbance of the sample without sodium salicylate.

The EC_{50} value, the effective concentration at which the OH scavenging effect being 50% was obtained by linear equation from graph plotted between scavenging percentage and essential oil concentration.

H_2O_2 scavenging assay

H_2O_2 scavenging activity was determined according to the method of Wang, *et al*. (2008) with some modifications. 1 ml of different concentrations of the essential oil (0-5000 µg/ml) in methanol was mixed with 1 ml of 0.1 mM H_2O_2 and followed by 100 µl of 3% ammonium molybdate solution, 10 ml of 2 M H_2SO_4 and 7 ml of 1.8 M KI. After that, the reaction mixture was titrated with 5 mM $Na_2S_2O_3$ until the violet color disappeared. The violet color results from the addition of 2% starch solution into the reaction mixture before titration. *L*-ascorbic acid was used as a positive control. The percent H_2O_2 scavenging activity was calculated as follows:

$$\text{Percent inhibition} = [(V_0-V_1)/V_0] \times 100\%$$

where V_0 is the volume of $Na_2S_2O_3$ solution used to titrate the blank (without the essential oil) and V_1 is the volume of $Na_2S_2O_3$ solution used to titrate the reaction mixture.

The EC_{50} value, the effective concentration at which the H_2O_2 scavenging effect being 50% was obtained by linear equation from graph plotted between scavenging percentage and essential oil concentration.

Reducing power assay

The reducing power of the essential oil was carried out according to the method of Tsai, *et al*. (2006) with some modifications. 2.5 ml of different concentrations of the essential oil in methanol (0-5000 µg/ml) was mixed with a mixture of 2.5 ml of 200 mM PBS (pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. The reaction mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was added with 2.5 ml of 10% w/v trichloroacetic acid, and then centrifuged at 200 rpm for 10 min. After that, 5 ml of the supernatant was added with 5 ml of methanol and 1 ml of 0.1% $FeCl_3$. The absorbance of the reaction mixture was measured at 700 nm against the blank, which did not contain the essential oil. The increment in the reaction-mixture absorbance indicates the increased reducing power of the sample. *L*-ascorbic acid was used as a positive control.

The essential oil concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against essential oil concentration.

Antibacterial activity

Bacterial strains

All bacterial strains used for this study were obtained as lyophilized cultures from Department of Medical Science, Ministry of Public Health Thailand. Three species of gram positive, i.e. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633 and one species of gram negative, i.e. *Escherichia coli* ATCC 25922 were used for this study.

Agar diffusion method

Microbial cultures for antimicrobial activity assay were freshly cultured on Tryptic Soy Broth (TSB) medium and incubated at 37°C for 24 hr. After that, the TSB medium was approximately adjusted to solution concentrations of 0.5 McFarland with 0.9% sterile normal saline solution. The mixtures were spread on Tryptic Soy Agar (TSA) plate with sterile cottons swab and allowed to dry. Sterilized paper filter discs with a diameter of 6 mm were impregnated with 20 µl of an essential oil solution (0-5000 µg/ml) in 5% v/v

dimethylsulphoxide (DMSO) dissolved in TSB and placed on the inoculated agar. The plates were left for 30 min at room temperature to allow the diffusion of the oil and then they were incubated at 37°C for 24 hr. Antimicrobial activity was evaluated by measuring the clear zone of inhibition against the test microorganisms. In this experiment, two controls were used, i.e. a control having microorganism without test material and a control having 5 μg/ml standard tetracycline solution in 5% v/v DMSO dissolved in TSB. The tetracycline solution was used as a positive control. Experimental tests were performed in triplicate and the developing inhibition zones were compared with those of reference disc.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) for antibacterial activity assay was determined by the broth dilution method (Karaman *et al*., 2003; Daud *et al*., 2005). The inocula of microorganisms were prepared from 24 hr broth cultures and suspensions were adjusted to 1 McFarland standard turbidity. The essential oil dissolved in 5% v/v DMSO in TSB was first diluted to the highest concentration (200 μg/ml) prior to being tested, and then serial two-fold dilutions were made in a concentration range from 200 to 1.56 μg/ml in 10 ml sterile test tubes containing nutrient broth. Solvent, antibiotic and microorganism controls were also analyzed. The mixture in each tube was incubated at 37°C for 24 hr. The MIC value of the essential oil was determined as the lowest concentration of the essential oil that completely prevent any turbidity or growth of the test organisms. All samples were tested in triplicate.

Antimycobacterial activity

Green Fluorescent Protein Reporter Microplate Assay (GFPMA) was applied for the determination of antimycobacterial activity of the essential oil (Collins *et al.*, 1998 and Changsen *et al*., 2003). The tested mycobacterial strain used in this study was *Mycobacterium tuberculosis* strain H37Ra. 5 μl of various concentrations of the essential oil in 5% DMSO was added into each well containing 45 μl of cell suspension (2×10^4 - 1×10^5 cfu/ml). The plate containing test samples was incubated at 37°C for 7 days. After that, the fluorescence signals were measured using SpectraMax M5 microplate reader in the bottom-reading mode with excitation at 485 nm and emission at 535 nm. The fluorescence signals on day zero were used as a background. The percentage of growth inhibition was calculated according to the following equation:

$$\text{Percent inhibition} = [1-(FU_T/FU_C)] \times 100$$

where FU_T was the fluorescence units of cells treated with the essential oil and FU_C was the fluorescence units of cells untreated with the essential oil.

The minimum inhibitory concentration (MIC) for antimycobacterial activity assay was defined as the lowest concentration at which the growth inhibition of the test organisms by the essential oil was found to be 90%. Isoniazid was used as a positive control and 0.5% DMSO was used as a negative control. All samples were tested in triplicate.

Statistical analysis

All experiments were repeated at least three times. Results were reported as mean ± S.D. EC_{50} values were also calculated. Statistical comparison was made with independent samples *t*-Test at the confidence interval of 95% (SPSS Statistics version 18).

RESULTS AND DISCUSSION

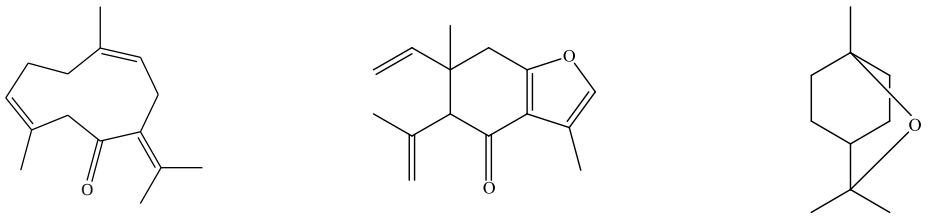
Chemical compositions of the essential oil

Essential oil obtained from the water distillation of fresh rhizome of *C. aeruginosa* was clear and pale yellow oil with the percent yields of 0.23 % v/w.

The chemical constituents of the essential oil, their peak area percentages and Kovats Indices (KIs) are compiled in Table 1, in order of their elution on the BPX 5 column.

A total of 22 compounds, corresponding to 95.39%, were identified. The essential oil was mainly dominated by oxygenated sesquiterpenes (42.85%) follow by sesquiterpene hydrocarbons (30.80%), oxygenated monoterpene (10.92%) and monoterpene hydrocarbons (10.82%). The three major components were germacrone (23.49%), curzerenone (11.78%) and 1,8-cineole (10.92%). The structures of major components in *C. aeruginosa* rhizome oil are shown in Figure 1.

Similar experimental results were previously reported by Sirat et al., 1998. Curzerenone and 1,8-cineole were reported as the major component in rhizome oil of *C. aeruginosa* from Malaysia. In addition, germacrone was reported as the major component in rhizome oil of *C. harmadii* (Dũng *et al*., 1997) and *C. leucorhiza* (Devi *et al*., 2012). However, our experimental results were differently apparent from those previously reported (Sirat *et al*., 1998). Our results showed the presence of germacrone in high amounts (23.49%) and the absence of camphor whereas the results of Sirat, Jamil and Hussain (1998) showed the high amounts of camphor (10.50%) and low amounts of germacrone (2.70%).



(a) (b) (c)

Figure 1. The three major components of essential oil from the fresh rhizomes of *C. aeruginosa*
(a) germacrone (b) curzerenone (c) 1,8-cineole

Table 1: Essential oil components of the fresh rhizome of *C. aeruginosa*

Chemical composition	Class[a]	KI[b]	Content(%)
α-Pinene	MH	939	1.75
β-Pinene	MH	979	9.07
1,8-Cineole	OM	1031	10.92
β-Elemene	SH	1390	1.79
E-Caryophyllene	SH	1419	0.70
γ-Muurolene	SH	1479	1.56
ar-Curcumene	SH	1480	6.71
β-Selinene	SH	1490	1.75
cis-β-Guaiene	SH	1493	3.37
α-Muurolene	SH	1500	0.94
β-Bisabolene	SH	1505	1.26
α-Bulnesene	SH	1509	0.86
γ-Cadinene	SH	1513	0.76
δ-Cadinene	SH	1523	9.14
Germacrene B	SH	1559	1.96
Spathulenol	OS	1578	0.50
Curzerenone	OS	1606	11.78
β-Atlantol	OS	1608	1.33
epi-α-Cadinol	OS	1640	2.63
α-Cadinol	OS	1654	1.15
Germacrone	OS	1693	23.49
8-Hydroxy-eremophilone	OS	1847	1.97

a: MH: Monoterpene hydrocarbon; OM: Oxygenated monoterpene;
SH: Sesquiterpene hydrocarbon; OS: Oxygenated sesquiterpene
b: Kovats index is determined relative to n-alkanes (C6–C24) on a BPX 5 column

Table 2: EC_{50} values of the essential oil from the fresh rhizome of *C. aeruginosa* in various antioxidant systems as compared with the positive control *L*-ascorbic acid[*]

Test	EC50 (μg/ml)	
	Essential oil	*L*-Ascorbic acid
DPPH radical scavenging assay	24.32 ± 2.13[a]	21.21 ± 2.29[a]
OH Scavenging assay	244.48 ± 21.24[a]	228.24 ± 16.09[a]
H_2O_2 Scavenging activity assay	4031.37 ± 188.46[a]	346.75 ± 42.79[b]
Reducing power assay	> 5000[a]	453.93 ± 13.78[b]

[*] Data are expressed as means ± SD (n = 3).
Means ± SD followed by the same letter for each experiment, within a row, are not significantly different ($P > 0.05$).

Antioxidant activity

The antioxidantive capacities of essential oil were elucidated by using $DPPH^{\bullet}$, $OH^{\bullet}$ and H_2O_2 scavenging assays as well as reducing power assay. EC_{50} values, which were corresponding to $DPPH^{\bullet}$, $OH^{\bullet}$ and $H_2O_2^{\bullet}$ scavenging assays were used to compare to its antioxidantive capacities. EC_{50} is defined as the effective concentration at which 50% of radicals are scavenged and the lower EC_{50} value reflects better protective action. The effectiveness of scavenging $DPPH^{\bullet}$ and $OH^{\bullet}$ radicals has no statistically significant difference between the essential oil *L*-ascorbic acid as seen in EC_{50} values listed in Table 2. The essential oil was less effective for scavenging H_2O_2 than *L*-ascorbic acid (EC_{50} = 4031.37 and 346.75 μg/ml, respectively). The high effectiveness towards $DPPH^{\bullet}$ and $OH^{\bullet}$ scavenging of essential oil may be due to the presence of nonphenolic constituents such as germacrone and curzerenone which can react with the stable $DPPH^{\bullet}$ and $OH^{\bullet}$ radicals by donating hydrogen atoms. Similar experimental results were previously reported (El-Massry, El-Ghorab and Farouk, 2002; Tepe *et al.*, 2004; Deba *et al*., 2008). El-Massry and co-workers (2002) stated that some essential oils rich in nonphenolic compounds also have antioxidant potentials.

In case of the H_2O_2 scavenging assay, H_2O_2 is not as very reactive as $OH^{\bullet}$ radical, and so it may react very slowly with the nonphenolic compounds in the essential oil as compared to *L*-ascorbic acid.

In reducing power assay, the essential oil did not show the capacity of reducing the Fe^{3+}/ferricyanide complex to the ferrous form. The results indicated that the components existing in the essential oil may be weaker reducing agents for Fe^{3+}metal ion.

Antibacterial activity

The result of antibacterial activity assays using agar diffusion technique showed that the essential oil has modulate activity against *E. faecalis* ATCC 29212 with MIC value of 6.25 μg/ml and weak activity against *B. subtilis* ATCC 6633 with MIC 50 μg/ml whereas it exhibited no activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 (Table 3). However, it is very difficult to attribute the biological effect of a total essential oil to one or a few active principles, because in addition to the major compounds, also minor compounds may make a significant contribution to the oil activity (Saha *et al*., 2013).

Antimycobacterial activity

The inhibitory effect of the essential oil against *Mycobacterium tuberculosis* strain H37Ra has been assessed by Green Fluorescent Protein Microplate assay (GFPMA). It was found that the essential oil from the fresh rhizome of *C. aeruginosa* possessed the inhibitory activity against *M. tuberculosis* strain H37Ra tested with MIC value of 2500 mg/ml. The positive control isoniazid for this test gave the MIC value of 0.023-0.046 μg/ml.

Table 3: Antibacterial activity and minimal inhibitory concentration (MIC) of the essential oil from the fresh rhizome of *C. aeruginosa* as compared with the positive control tetracyclin

Test bacteria	Zone of inhibition (mm)[a]		MIC (μg/ml)	
	Essential oil[b]	Tetracyclin[c]	Essential oil	Tetracyclin
E. coli ATCC 25922	-	10 ± 0.8	-	< 0.04
S. aureus ATCC 29213	-	11 ± 0.8	-	< 0.04
B. subtilis ATCC 6633	9 ± 0.2	8.7 ± 0.5	50	< 0.04
E. faecalis ATCC 29212	11 ± 0.1	8.7 ± 0.6	6.25	< 0.04

Each value is expressed as means ± standard deviation (n=3)

[a] include disc diameter (6.0 mm)

[b] concentration of solution tested (200 μg/ml)

[c] concentration of solution tested (5 μg/ml)

- no inhibition

CONCLUSION

The results of the present study showed that the essential oil from the fresh rhizome of *C. aeruginosa* was rich in oxygenated sesquiterpenes, i.e. germacrone. With regard to the scavenging ability to DPPH and OH radicals, the essential oil showed strong antioxidant activity as compared with *L*-ascorbic acid. In addition, the essential oil exhibited strong inhibitory activity against *E. faecalis* ATCC 29212, but gave weak inhibitory activity against *M. tuberculosis* strain H37Ra. This is the first report of antimycrobacterial activity of essential oil from the fresh rhizome of *C. aeruginosa*. The results suggest that the essential oil from the fresh rhizome of *C. aeruginasa* might be a potential source of natural antioxidant and antibacterial substances.

ACKNOWLEDGMENTS

The authors are grateful to Assist. Prof. Dr. Thaya Jenjittikul, Department of Plant Science, Faculty of science, Mahidol University for her kindness, meaningful advice and valuable information.

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