CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OIL OF *CURCUMA PHAEOCAULIS* VALETON

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Abstract

Chemical composition, antioxidant and antibacterial activities of the essential oil of *Curcuma phaeocaulis* were analysed. In the aerial part, 22 compounds account for 69.23% of the total essential oil and authenticated, while in the underground part, 32 compounds account for 84.28% of the total essential oil and authenticated. Among the compounds detected, α-pinene, camphene, eucalyptol, camphor, borneol, β-tumerone, epi-curzerenone were the main compounds of volatile oil in both parts of *Curcuma phaeocaulis*. In addition, various lipid antioxidant properties by peroxidation assays, inoxidizability and antibacterial action were assessed. The result may be considered as a baseline study for the further development and application of *Curcuma phaeocaulis*.

Introduction

Curcuma is an important genus belonging to Zingiberaceae and this plant group is widely used in medical, food, chemical and ornamental. There were about 120 plant species in this family (Záveská 2012). Out of them 10 species of *Curcuma* were found in China (Xiao *et al.* 1997, Wu 2008, Chen and Xia 2010). There are books which recorded that *Curcuma phaeocaulis* was used as Chinese traditional herbal medicine with a history of more than a thousand years. In traditional Chinese medicine (TCM), Rhizoma Curcumae was the rhizomes of *C. phaeocaulis*. Recent studies have shown that people use some extract of *C. phaeocaulis* to process activity experiment and obtained the predictive effect (Hou *et al.* 2015). Lipids belonging to a group of naturally occurring molecules was used as diet in the daily lives. Sometimes lipids might occur some undesirable direction such as oxidation and lipolysis (Kinderlerer and Johnson 1992). Lipid peroxidation decrease many food products nutritional value and food safety. Moreover, it also changes foods' color, quality and taste. Food products under environmental exposure gave rise to a free radical process, and might give rise to auto-oxidation of polyunsaturated lipids. Assuming the use of antioxidants that can inhibit the activity of free radical oxidation is practical in preventing grease deterioration.

In order to prevent oxidative degradation of foods, antioxidants as a food additive are widely used in food product (Jiang and Xiong 2016). Many antioxidants are synthetic chemicals, which have serious side effects, although they usually have a strong radical scavenger (Imaida *et al.* 1983). Therefore, the antioxidants which obtained naturally become an innovation and research focus in recent years. *C. longa* (turmeric) is a perennial herb, which is used as folk medicine in many disease treatments for a long time. More than that, its oils often is used as spice, textiles and cosmetics (Mahendra and Bisht 2011). In India, turmeric rhizomes are used as medicine too. Previously many studies showed that turmeric is used in clinical treatment to against cancer, AIDS

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and so on (Prasad *et al.* 2015, Azuine and Bhide 2016, Feng *et al.* 2017). The volatile compounds extracted from *Curcuma* had a good biological activity (Hwang *et al.* 2000, Wilson *et al.* 2005, Sathishkumar *et al.* 2010, Jena *et al.* 2017).

In the present study, the essential oils, chemical composition and functional activities in both aerial- and underground part of *C. phaeocaulis* were analysed. This work will provide a good guidance for the use of *C. phaeocaulis*.

Materials and Methods

Both aerial- and underground part of *Curcuma phaeocaulis* were collected from Sichuan Province, China. The plant materials were identified by Prof. Ruiwu Yang from the Department of Botany of Sichuan Agricultural University and the specimens were deposited in Sichuan Agricultural University. The experiment was carried out in the Laboratory of Resource Plant, Department of Botany, College of Life Science, Sichuan Agricultural University.

The aerial- and underground part of *C. phaeocaulis* were separated and washed. Then, each part was air dried in dark at room temperature. The dried materials were ground to thinly grated in a grinder and sun dried. According to the aerial- and underground part, respectively pulverized into a fine powder. Gas chromatography mass spectrometry (GCMS-QP2010) was used to analyze chemical components. The processed materials were placed in a Soxhlet extraction apparatus for 5 hrs and extracted three times with hexane. The extracted oils with a light-yellow color and the oils yield 1.03% for aerial part and 3.34% for underground part. The extracted oils were dried over anhydrous sodium sulfate and stored at 4°C.

The GCMS technique used to analyze the chemical composition of essential oils which was extracted from both aerial- and underground parts of *C. phaeocaulis* materials. The GCMS analyses were performed using gas chromatography mass spectrometry instrument island ferry company GCMS-QP2010 and equipped with a Perkin Elmer Elite-5MS capillary column (5% phenylmethyl siloxane, length 30 m × inner diameter 0.25 mm×film thickness 0.25 μm). Separately, the injector and interphase were set at the same temperatures at 280°C, the ion source temperatures were maintained at 230°C and selective mass detector temperatures was 150°C. Helium was the carrier gas and the flow rate is 1.0 ml/min. Different parts of *C. phaeocaulis* materials had different oven temperature. The essential oil from underground part, the GC oven temperature was kept at 60°C for 1 min, and then increase programmed from 6 to 185°C at the rate of 1.5°C/min and kept at 185°C for 1 min, and then the GC oven temperature was kept at 60°C for 1 min, and then increase programmed from 18 to 275°C at the rate of 9°C/min and kept at 275°C for 2 min. The essential oil from aerial part, the GC oven temperature was kept at 80°C for 0 min, and then increase programmed from 8 to 280°C at the rate of 10°C/min and kept at 280°C for 40 min. Using the normalization method from the GC peak areas to computed the percentage composition of the oils, and all results were performed in triplicate and averaged.

In the free radicals 2,2-diphenyll-picrylhydroazyl (DPPH) radical scavenging assay, the method of Yang *et al.* was followed with some modifications (Yang *et al.* 2009). In a assay, 2.0 ml different concentrations gradient (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml in 95% ethanol) and 2.0 ml DPPH solution (0.1 mmol/l in 95% ethanol) were mixed in the reaction solution in the tubes. After mixing, the solution was left for 60 min at room temperature. The DPPH radical inhibition could be measured by using a spectrometer (UV-1750, Shimadzu, Kyoto, Japan) reader in 517 nm. This experiment was repeated three times and then the data needed to average. Vc was treated as control. The capability to scavenge the DPPH radical was given by:

Scavenging ability (%) = $(1 - A_{sample}/A_{DPPH}) \times 100$

where the absorbance value of A_{sample} was mixed solution of DPPH solution and related tested samples, and A_{DPPH} was blank consisted of only ethanol.

In the hydroxyl radicals scavenging activity assay, the method of Jiang *et al.* was as followed with some modifications (Jiang *et al.* 2015). In a assay, 1.0 ml H_2O_2 (0.1 %,), 1.0 ml of $FeSO_4$ (0.75 mmol/l), 2.0 ml of PBS (pH 7.4) and 2.0 ml different concentrations gradient (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml in 95% ethanol) sample solution were mixed in the reaction solution. After mixing, the solution was left for 60 min at 37°C. The Hydroxyl radicals scavenging activity could be measured by using a spectrometer (UV-1750, Shimadzu, Kyoto, Japan) reader in 510 nm. This experiment was repeated 3 times and then the data needed to average. Vc is as a control. The capability to scavenge the hydroxyl radical was given by:

Scavenging ability (%) = $(A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100$

where, the absorbance value of A_{sample}, $A_{control}$ and A_{blank} were presence of sample, blank without of H_2O_2 and the absence of sample, respectively.

In the superoxide radical scavenging ability assay was assessed following the method of Liu *et al.* (2009) with some modifications. In an assay, 1.0 ml Tris-HCl (16 mmol/l, pH 8.0) and 1.0 ml sample solution (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml in 95% ethanol) were prepared in first. And then 468 µmol/l NADH, 60 µmol/l PMS and 468 µmol/l NBT were prepared in1.0 mL Tris-HCl buffer. After mixing, the solution was left for 5 min at 25°C, the superoxide-radical scavenging ability could be measured by using a UV-spectrophotometer (UV-1750, Shimadzu, Kyoto, Japan) reader in 560 nm. This experiment was 3 times repeated and then the data needed to average. Vc was treated as control. The capability to scavenge the hydroxyl radical was given by:

Scavenging ability (%) = $(1 - A_{sample}/A_{blank}) \times 100 \%$

where, the average absorbance value about A_{sample} and A_{blank} were the sample and the absence of sample, respectively.

The measurement of FRAP was assessed following the method of Yuan *et al.* (2005) used with some modifications. In a assay, 2.5 ml PBS (0.2 mol/l, pH 6.6), 2.5 ml sample solution (0, 0.1, 0.5, 1.0, 1.5, 2.0 mg/ml in 95% ethanol) and 1.0 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ (1%, w/v) were mixed in the reaction solution. After mixing, the solution was left for 20 min at 50°C. The mixed solution was centrifuged at 3 000 r/pm for 10 min and it was kept for   cooling. Next 2.5 ml trichloroacetic acid [TCA] (10%, w/v) was added in the solution. In a test tube, the 0.5 ml ferric chloride $[FeCl_3]$ and 2.5 ml of absolute ethyl alcohol were mixed with the 2.5 ml supernatant. After mixing the mixture it was kept for 10 min. Then the absorbance was measured by using a UV-spectrophotometer at 700 nm. Increase in absorbance of the reaction mixture indicated increase in reducing power. This experiment was repeated for 3 times and was averaged. Vc was treated as a control.

Agar well diffusion method was used to evaluate the antimicrobial activity of zedoary turmeric oil extracted from the samples, namely. *Staphylococcus aureus* and *Bacillus cereus* (two Gram-positive bacterial strains) and *Salmonella* and *Escherichia coli* (two Gram-negative bacterial strains) were used to test it. All strains obtained from the Department of Biochemistry and Molecular Biology, Sichuan Agriculture University.

Nutrient agar (18 g) was dissolved in distilled water for 1.0 litre and the pH was adjusted to 7.0 in the nutrient agar aqueous solution. Before test, the autoclave was used for sterilizing all glass wares and media in 15 psi at 121°C for 21 min. The required concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) were needed to give appropriate dilutions about the essential oil which were accurately weighted and dissolved in sterile 95% ethanol. The prepared solutions were stored at −20°C. The agar well diffusion method showed the antimicrobial activity about the turmeric oil of *C. phaeocaulis*. In a laminar airflow, the inoculation was carried out in all the trials . The

inoculated nutrient agar plate needed to cool before inoculation. Swabbing method was used to inoculate the bacterial strains. Holes punch a diameter of 6 mm and a spacing of 4 cm were prepared in the medium using sterilized cork drillers to prepare four uniform holes in each well. The base of each hole was sealed with a drop of molten nutrient agar. To fill these wells the micropipette was used with 50 µl of essential oil and let them diffuse for 40 min. All the inoculated plates were incubated in the incubator at 37°C for 24 hrs to determine the antimicrobial activity. The diameter of the inhibition zone produced by the essential oil around the well indicated the antimicrobial activities. This experiment was also carried out 3 times for each sample to evaluate of antimicrobial activity.

The bacteriostatic circle standard (Paulo *et al.* 2010): inhibitory zone diameter greater than 20 mm, supersensitive; 15 - 20 mm, hypersensitive; 10 - 15 mm, the sensitive; 7 - 9 mm, low sensitive, less-than 7 mm, resistant.

Results and Discussion

The chemical compostion of the essential oil of aerial part (AP) and underground part (UP) of *Curcuma phaeocaulis* is presented in Table 1. About 32 components were identified in the underground part essential oil representing about 84.28% of the total amount and 22 components were identified in aerial part essential oil constituting about 69.23% of the total weight. The major components were germacron (16.89%), epi-curzerenone (16.35%), β-tumerone (6.47%), camphor (5.11%), borneol (5.03), eucalyptol (4.96%), ar-tumerone (4.46%) and ar-curcumene (4.11%). In underground part essential oil of *C. phaeocaulis*, were eucalyptol (28.68%), camphor (4.31%), epi-curzerenone (3.68%), borneol (3.66%), β-Pinene (3.27%), and L-linalool (3.02%) in aerial part essential oil of *C. phaeocaulis*.

It is interesting to note that a major component in the essential oil germacron (16.89%) and epi-curzerenone (16.35%) from underground part while a very minor quantity in the essential oil germacron (0.86%) and epi-curzerenone (3.68%) of the aerial part. The contents of α-pinene (2.34%), β-pinene (3.27%), D-limonene (2.34%), eucalyptol (28.68%) and β-elemene (2.72%) in underground part are very less and a half of the amount found in aerial part.

In 517 nm, the DPPH as a stable free radical has a characteristic absorption maximum. As a tool, it is used to assessing the DPPH free radical scavenging activity of antioxidants and is widely accepted (Hua *et al.* 2014, Milella *et al.* 2014, Xie *et al.* 2014, Musa *et al.* 2016). DPPH free radicals are scavenged by the fact that natural compounds can transfer electrons or hydrogen atom to DPPH (Naik *et al.* 2003). During this reaction, the absorbance decreases and the solution changes from purple to pale yellow. Compared with Vc (controlling standard), the results showed that the different concentrations of essential oil had different DPPH scavenging abilities (Fig. 1). The essential oil of underground part showed stronger activity than the essential oil of dry aerial part. 1.0 mg/ml, scavenging ability on DPPH radical are keeping balance at underground part (86.37%) and aerial part (74.16%). The underground part essential oil was close to Vc. In this study, the essential oil showed a high scavenging ability in all concentration gradients. This finding was found to be similar with report of Zaeoung *et al.* (2005).

The ferrous ion chelating effect of the extracts by Fe^{+2} ferrozine test is presented shown in Fig. 2. The red decreased, the reason is that when in the presence of ion chelating substances, the ferrozine can quantitatively form complex with Fe2+ and the complex formation is disrupted. A higher absorbance value means that the reduction capacity of the sample is stronger, and according to the reduction capacity of the compound, the color of the mixture will change from yellow to blue or green. Among the tested substances, essential oil of underground part showed higher Fe^{2+} chelating ability than that of aerial part. When the concentration is 2.0 mg/ml, the ferrous ion chelating capacity is the strongest and close to Vc.

Table 1. Chemical composition of the essential oil of aerial part (AP) and underground part (UP) of *Curcuma phaeocaulis.*

No.	Compound	Mol. Form	UP (%)	AP (%)	Identification
1	α-pinene	$C_{10}H_{16}$	1.02	2.34	MS, RI, co-GC
2	Camphene	$C_{10}H_{16}$	2.03	2.08	MS, RI, co-GC
3	β-phellandrene	$C_{10}H_{16}$	-	0.48	MS, RI, co-GC
4	β-pinene	$C_{10}H_{16}$	0.62	3.27	MS, RI, co-GC
5	β-myrcene	$C_{10}H_{16}$	-	0.33	MS, RI, co-GC
6	Carene	$C_{10}H_{16}$	0.11	-	MS, RI
7	D-limonene	$C_{10}H_{16}$	0.06	2.34	MS, RI
8	Limonene	$C_{10}H_{16}$	0.56	-	MS, RI
9	Eucalyptol	$C_{10}H_{18}O$	4.96	28.68	MS, RI
10	Linalool	$C_{10}H_{18}O$	0.07	-	MS, RI
11	L-linalool	$C_{10}H_{18}O$	-	3.02	MS, RI
12	Camphor	$C_{10}H_{16}O$	5.11	4.31	MS, RI
13	Isobornoel	$C_{10}H_{18}O$	2.82	1.08	MS, RI
14	Borneol	$C_{10}H_{18}O$	5.03	3.66	MS, RI
15	Terpinene	$C_{10}H_{18}O$	0.59	-	MS, RI
16	Terpineol	$C_{10}H_{18}O$	0.58	0.11	MS, RI
17	2-pinen-10-ol	$C_{10}H_{18}O$	2.53	1.08	MS, RI
18	δ-elemene	$C_{15}H_{24}$	1.59	-	MS, RI
19	β-elemene	$C_{15}H_{24}$	0.42	2.72	MS, RI
20	α-cedrene	$C_{15}H_{24}$	0.89	-	MS, RI
21	β-cedrene	$C_{15}H_{24}$	0.36	-	MS, RI
22	Caryophyllene	$C_{15}H_{24}$	0.81	1.93	MS, RI
23	γ-elemene	$C_{15}H_{24}$	0.43	-	MS, RI
24	ar-curcumene	$C_{15}H_{24}$	4.11	-	MS, RI
25	α-longipinene	$C_{15}H_{24}$	0.22	-	MS, RI
26	α-gurjunene	$C_{15}H_{24}$	0.17	-	MS, RI
27	Elemol	$C_{15}H_{26}O$	1.62	0.72	MS, RI
28	Caryophyllene oxide	$C_{15}H_{24}$	-	1.76	MS, RI
29	α-elemene	$C_{15}H_{24}$	0.78	2.01	MS, RI
30	α-caryophyllene	$C_{15}H_{24}$	0.46	-	MS, RI
31	β-tumerone	$C_{15}H_{20}O$	6.47	2.01	MS, RI
32	Cedrol	$C_{15}H_{26}O$	1.03	-	MS, RI
33	Epi-curzerenone	$C_{15}H_{28}O_2$	16.35	3.68	MS, RI
34	Eudesmol	$C_{15}H_{26}O$	1.13	-	MS, RI
35	Ar-tumerone	$C_{21}H_{20}O_6$	4.46	0.76	MS, RI
36	Germacron	$C_{15}H_{22}O$	16.89	0.86	MS, RI
	Total		84.28	69.23	

The retention index (RI) was calculated using a homologous series of n-alkanes C10-C21. Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector. [a]Co-GC: Co-injection with an authentic sample.
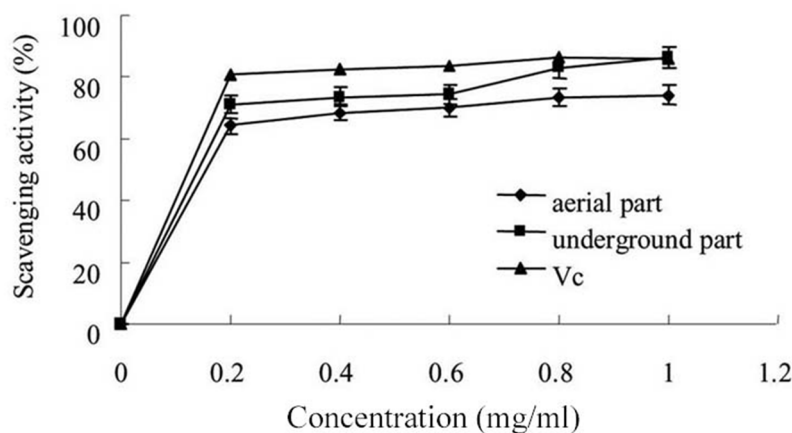
Fig. 1. Radical scavenging effect of *Curcuma phaeocaulis* essential oil on 2,2'-diphenyl-1-picrylhydrazyl radical.
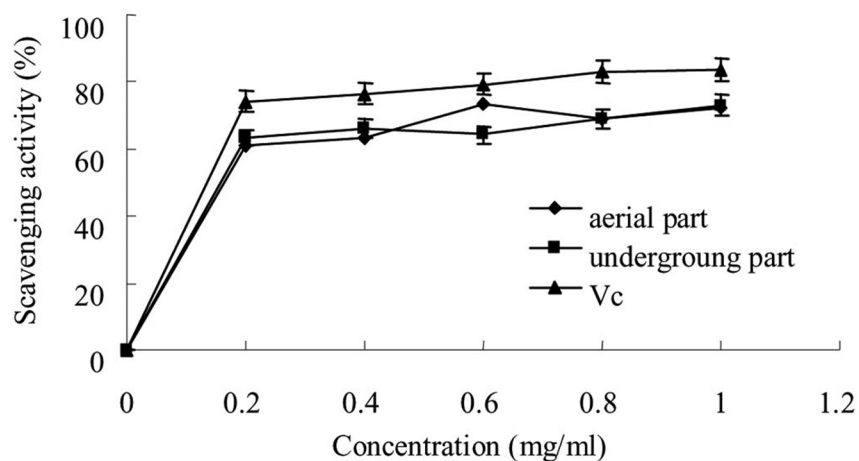

Fig. 2. Radical scavenging effect of *Curcuma phaeocaulis* essential oil on hydroxyl radical.

Hydroxyl radicals are extremely potent oxidants with the ability to cross cell membranes, readily react with most biomolecules, and may damage cells and tissues by Fenton reaction to produce hydroxyl radicals. To protect the tissues from oxidative damage, removal of hydroxyl radicals is a good way (Galano *et al.* 2011, Kryston *et al.* 2011). The scavenging activity is concentration dependent, with sample concentrations ranging from 0 to 1.0 mg/ml (Fig. 3). When the concentration reached 1.0 mg/ml, the hydroxyl radical scavenging capacity remain balance in the underground part of 73.12% and aerial part of 72.19%. The results showed that the essential oil had strong ability to scavenge hydroxyl radical, and the underground part was close to aerial part.

In different reactive oxygen species, the superoxide anion generated first. It acts as a precursor of singlet-oxygen and hydroxyl radicals, has greater oleophilic and oxidative ability. It is necessary to scavenge superoxide radical. The superoxide radical can induce arthritis and

Alzheimer's disease (Mateen *et al.* 2016), the reason is the superoxide radical can induce lipid peroxidation and cause extensive cellular damage by contributing to the production of highly reactive radical species (Athukorala *et al.* 2006). Fig. 4 shows the superoxide anion-scavenging activity. The PMS/NADM system can assay in the reduction of NBT, and it is used to measure the superoxide anion-scavenging ability. At all tested concentrations, the scavenging activity of essential oils followed a dose-dependent pattern. In the underground part and aerial part, the superoxide radical scavenging effects were 83.92 and 76.21%, respectively at the same concentration of 1.0 mg/ml. In addition, the results showed that the essential oil had an obvious scavenging activity of superoxide radical.

The essential oil extract in underground part and aerial part of *C. phaeocaulis* was suitable for testing the antibacterial activities against Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacterial strains (*Salmonella* and *Escherichia coil*). Tables 2 and 3 showed that the essential oil of underground part had stronger activity than that of aerial part. When concentration reached 1.0 mg/ml, the aerial part essential oil reached a very sensitive to *Escherichia coli* , and the underground part to *Staphylococcus aureus*, *Salmonella* and *Escherichia coil*.

Table 2. Antibacterial activity of aerial part essential oil of *Curcuma phaeocaulis*.

Test bacteria	Inhibition zone (mm)				
	0.2 (mg/ml)	0.4 (mg/ml)	0.6 (mg/ml)	0.8 (mg/ml)	1.0 (mg/ml)
Staphylococcus aureus	7.93 ± 0.02a	9.32 ± 0.03b	11.00 ± 0.07c	13.56 ± 0.04d	14.67 ± 0.05e
Bacillus cereus	7.63 ± 0.05a	7.65 ± 0.02a	9.21 ± 0.04b	10.21 ± 0.08c	11.34± 0.08d
Salmonella	8.23 ± 0.01a	8.85 ± 0.05b	8.86 ± 0.04b	9.12 ± 0.03c	9.43 ± 0.02d
Escherichia coli	9.16 ± 0.05a	10.44 ± 0.08b	12.19 ± 0.07c	14.21 ± 0.08d	15.34 ± 0.02e

Table 3. Antibacteria activity of the underground part of *Curcuma phaeocaulis*.

Test bacteria	Inhibition zone (mm)				
	0.2 (mg/ml)	0.4 (mg/ml)	0.6 (mg/ml)	0.8 (mg/ml)	1.0 (mg/ml)
Staphylococcus aureus	9.23 ± 0.03a	11.29 ± 0.03b	12.96 ± 0.04c	14.12 ± 0.04d	15.89 ± 0.08e
Bacillus cereus	9.17 ± 0.02a	12.02 ± 0.04b	14.21 ± 0.03c	14.36 ± 0.04d	14.38 ± 0.05d
Salmonella	11.27 ± 0.02a	12.43 ± 0.05b	13.26 ± 0.03c	15.18 ± 0.02d	15.21 ± 0.02d
Escherichia coli	11.39 ± 0.05a	11.41 ± 0.02a	13.26 ± 0.04b	15.29 ± 0.08c	16.37 ± 0.02d

From GC-MS data, it was clear that the major constituent's amount of epi-curzerenone and germacron in aerial part essential oil is less than that of the underground part. The presence of the compounds in major quantity might be due to the activity of underground part higher than aerial part. The lack of antioxidant activity during growth indicated that the beneficial pharmacological activity was bound to decrease. In other words, the underground part of *C. phaeocaulis* should be the first choice for medical, domestic and other purposes.

It is well known that the active principle in turmeric is a group of phenolic compounds including curcumin which has antioxidant activity (Kocaadam and Şanlier 2017). The acurcuminoids of turmeric has a strong antioxidant activity in some report (Barclay *et al.* 2000, Zaeoung *et al.* 2005, Nevin 2017). The present finding clearly showed that the essential oil form

turmeric is an effective antioxidant. The main component of essential oil is curcumenol and alpha-zingiberene. These compounds may have synergistic or additional effects on total antioxidant activity. Beyond that, it could be possible that the compounds might play a role in synergistic effect with other compounds. In present study, the essential oil from aerial part and underground part are answerable to the observed antioxidant activity of *C. phaeocaulis*. The results showed that the essential oils from the underground part exhibited higher antioxidant activity than those from the aerial part ones. From the comparison of aerial part and underground part which have different physical states in the same species, the antioxidant properties in underground part showed higher accumulation.
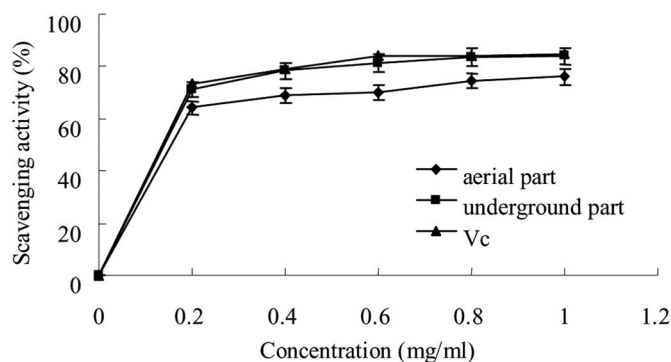

Fig. 3. Radical scavenging effect of *Curcuma phaeocaulis* essential oil on superoxide anion.
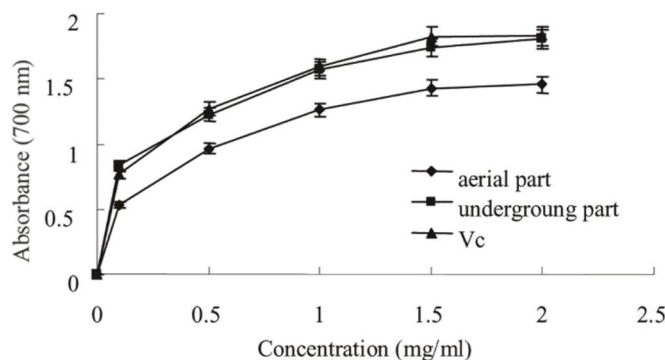

Fig. 4. Fe^{2+} chelating effect of *Curcuma phaeocaulis* essential oil.

It may be concluded that the antibacterial activities of essential oil have medicinal values. Thus, the essential oil could be utilized as an alternative source of the useful antimicrobial drugs. It could be recommended that *C. phaeocaulis* are highly important on the basis of their medicinal values. Along with the tested strains (*Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* and *Escherichia coil*), other strains could be also used for further investigations for its broad aspect importance.

Acknowledgments

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