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Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*

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Abstract

Curcuma zedoaria (Berg.) Rosc. (Zingiberaceae) has long been used as a folk medicine. The essential oil of its dried rhizome was isolated using simultaneous steam-distillation and solvent-extraction apparatus and its fractions were prepared by silica gel column chromatography. Totally, 36 compounds were identified in the essential oil, including 17 terpenes, 13 alcohols and 6 ketones. The yields of Fractions 2 and 3 were 83.66 and 10.71%, respectively. Epicurzerenone and curzerene were found in the first and second highest amounts (24.1 and 10.4%). At 20 mg ml^{-1}, the essential oil of *C. zedoaria* was moderate to good in antioxidant activities by three different methods, good in reducing power and excellent in scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical but low in chelating effect on ferrous ion. However, after fractionation, with regard to all antioxidant properties assayed, fraction 4 showed consistently better effects than the essential oil did. The compound in fraction 4 responsible for better antioxidant properties might be 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone.

Keywords: *Curcuma zedoaria*; Essential oil; Antioxidant activity; Reducing power; Scavenging effect; Chelating effect

1. Introduction

Curcuma zedoaria (Berg.) Rosc. (Zingiberaceae), also called Er-chu in Chinese, has long been used as a folk medicine. Traditionally, the dried rhizome of *C. zedoaria* is selected to make drinks or to be extracted as medicine. It has been reported that the boiling water extracts of *C. zedoaria* had a moderate antimutagenic activity against benzo[a]pyrene (Lee & Lin, 1988). Some hepatoprotective sesquiterpenes have been isolated from the aqueous acetone extracts of *C. zedoaria* rhizome. The major sesquiterpene compounds, including furanodiene, germacrone, curdione, neocurdione, curcumenol, isocurcumenol, aerugidiol, zedoarondiol, curcumenone and curcumin, were found to show potent protective effect on D-galactosamine/lipopolysaccharide-induced liver injury in mice (Matsuda, Ninomiya, & Yoshikawa, 1998). In a crude ethanolic extract of *C. zedoaria* rhizome, it has been found to have inhibitory activity against OVCAR-3 cells (a human ovarian cancer cell line). In addition, in the ethanolic extract, three identified active curcuminoids, including curcumin, demethoxycurcumin and bisdemethoxycurcumin, were found to possess this inhibitory activity (Syu et al., 1998). However, natural curcuminoids were also isolated from *C. longa* and showed reducing and antioxidant activities (Ruby, Kuttan, Babu, Rajasehnaran, & Kuttan, 1995).

To our knowledge, however, little information is available about the constituents of the essential oil of *C. zedoaria* rhizome and its antioxidant properties. Our objective was to extract the essential oil from the dried rhizome of *C. zedoaria* using a simultaneous steam-distillation and solvent-extraction method, and to fractionate the essential oil thus obtained into several fractions using silica gel column chromatography, and then identify the components in the essential oil and its fractions by gas chromatographic retention indices, mass

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spectra and authentic compounds. In addition, antioxidant properties of the essential oil and its fractions, including antioxidant activities, reducing power, scavenging and chelating effects, were determined.

2. Materials and methods

2.1. Plant material

The dried rhizome of *C. zedoaria*, imported from Sichuan Province, the People's Republic of China, was purchased from a traditional Chinese pharmacy in Changhwa, Taiwan. The sample was ground to 20 mesh in a comminution mill (Retsch Ultra Centrifugal Mill and Sieving Machine, Type ZM1, Haan, Germany), and the ground sample thus obtained was stored in the dark at −20 °C before use.

2.2. Isolation and fractionation of essential oil

Ground sample (100 g) was placed in a modified Likens-Nickerson apparatus. The solvent mixture (50 ml) of *n*-pentane and diethyl ether (column distilled, 1:1, v/v, Merck, Darmstadt, Germany) was used as an extractant. The simultaneous steam-distillation and solvent-extraction was allowed to proceed for 2 h, and the extract thus obtained was dried over anhydrous Na_2SO_4 (Merck) and filtered through Whatman No. 1 filter paper. The filtered extract was then concentrated at 40 °C to dryness using a Vigreux column (i.d. 1.5×100 cm, Tung Kawn Glass Co., Hsinchu, Taiwan) and the resulting essential oil was weighed and stored at −20 °C prior to chemical analyses. The isolation of the essential oil was carried out in triplicate.

The yellowish-brown essential oil was applied onto a silica gel-60 (55 g, 3–200 μm, Merck) column (i.d. 1.5×50 cm) and eluted sequentially with various solvents (100 ml each), *n*-pentane, *n*-pentane-diethyl ether mixture (75/25, 50/50 and 25/75, v/v) and diethyl ether, respectively. The corresponding fractions, 1–5, were obtained and then dried, concentrated and stored as described earlier. For analyses of antioxidant properties, the essential oil and its fractions were dissolved in methanol (Merck) to a concentration of 50 mg ml^{-1}.

2.3. Chemical characterization

Constituents in the essential oil and its fractions were analysed using a Hewlett-Packard 6890 gas chromatograph (GC) coupled to a HP 5973A MSD mass spectrometer (EI mode, 70 eV). A CP-Wax 52CB fused silica capillary column (i.d. 0.25 mm×60 m, 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands) was used and interfaced directly into the ion source of the MSD. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. The column temperature was programmed from 40 to 220 °C at 3 °C min^{-1}. Temperatures for GC injector and GC–MSD interface were 250 and 265 °C, respectively.

Components were identified on the basis of gas chromatographic retention indices, mass spectra from Wiley MS Chemstation Libraries (6th ed., G1034, Rev. C.00.00, Hewlett-Packard, Palo Alto, CA) and the literature (Adams, 1995; Jennings & Shibamoto, 1980). Some components were further identified using authentic compounds, which were commercially available. The relative amount of each individual component of the essential oil and its fractions was expressed as the percentage of the peak area relative to total peak area. Kovats indices were calculated for each separate component against *n*-alkane standards (C_8–C_{25}, Alltech Associates, Deerfield, IL) according to Schomberg and Dielmann (1973).

2.4. Antioxidant activity by 1,3-diethyl-2-thiobarbituric acid method

The antioxidant activity was determined by the 1,3-diethyl-2-thiobarbituric acid (DETBA) method (Furuta, Nishiba, & Suda, 1997; Suda, Furuta, & Nishiba, 1994). To 50 μl of the essential oil or its fractions (1∼20 mg ml^{-1}), in methanol, were added 50 μl of linoleic acid emulsion (Sigma Chemical Co., St. Louis, MO, 2 mg ml^{-1} in 95% ethanol). The mixture was incubated in an oven at 80 °C for 60 min, and cooled in an ice bath. The mixture was sequentially treated with 200 μl of 20 mM butylated hydroxytoluene (BHT, Sigma), 200 μl of 8% sodium dodecyl sulphate (SDS, Merck), 400 μl of deionised water, and 3.2 ml of 12.5 mM DETBA (Aldrich Chemical Co., Milwaukee, WI) in sodium phosphate buffer (pH 3.0, Wako Pure Chemical Co., Osaka, Japan). The mixture was thoroughly mixed, placed in an oven at 95 °C for 15 min, and then cooled in an ice bath. After 4 ml of ethyl acetate were added, the mixture was mixed and centrifuged at 1000×*g* at 20 °C for 15 min. Ethyl acetate layer was separated and its absorbance was measured in a Hitachi 650-40 spectrofluorometer (Tokyo, Japan) with fluorescence excitation at 515 nm and emission at 555 nm. The antioxidant activity was expressed as percent inhibition of lipid peroxidation, with a control containing no sample being 0%. A higher percentage indicates a higher antioxidant activity. Butylated hydroxyanisole (BHA), α-tocopherol and ascorbic acid were used as controls.

2.5. Antioxidant activity by thiobarbituric acid-reactive substance method

A modified thiobarbituric acid-reactive substances (TBARS) method was used to measure the antioxidant activity of the essential oil and its fractions in terms of

inhibition on lipid peroxidation (Tsuda, Osawa, Nakayama, Kawakishi, & Ohshima, 1993). Liposome suspension, consisting of 300 mg of lecithin (Sigma) and 30 ml of 20 mM sodium phosphate buffer (pH 7.4), was prepared in a sonicator (Ultrasonik 110T, NEY, Barkmeyer Div., Yucaipa, CA) for 2 h. The essential oil or its fractions (1–20 mg ml^{-1}) in methanol (0.5 ml) was mixed with a mixture of the sonicated solution (2 ml, 10 mg liposome ml^{-1}), $FeCl_3$ (0.1 ml, 25 mM), ascorbic acid (0.1 ml, 25 mM), and sodium phosphate buffer (1.2 ml, 20 mM, pH 7.4). The resulting mixture was incubated for 1 h at 37 °C and 1 ml of BHT (20 mg ml^{-1}), 2 ml of TBA (1%, w/v) and 1 ml of trichloroacetic acid (TCA, 2.8%, w/v, Wako) were added. The reaction mixture was incubated for 20 min at 100 °C and then cooled to room temperature. The absorption of the mixture was measured at 532 nm in a Hitachi U2001 spectrophotometer. The antioxidant activity was expressed as percent inhibition of lipid peroxidation, with a control containing no sample being 0%. A higher percentage indicates a higher antioxidant activity. BHA, α-tocopherol and ascorbic acid were used as controls.

2.6. Antioxidant activity by the conjugated diene method

The antioxidant activity was also determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). The essential oil or its fractions (1–20 mg ml^{-1}) in methanol (100 μl) was mixed with 2 ml of 10 mM linoleic acid emulsion (pH 6.5) in test tubes and placed in the dark at 37 °C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol in deionised water were added, and the absorbance of the mixture was measured at 234 nm in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows: AOA (%) = [(ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control] × 100%. An AOA value of 100% indicates the strongest antioxidant activity. BHA, α-tocopherol and ascorbic acid were used as controls.

2.7. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). The essential oil or its fractions (1–20 mg ml^{-1}) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% TCA were added, the mixture was centrifuged at 200 × *g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm in a Hitachi U-2001 spectrophotometer. A higher absorbance indicates a higher reducing power. BHA, α-tocopherol and ascorbic acid were used as controls.

2.8. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical

The scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992). The essential oil or its fractions (1–20 mg ml^{-1}) in methanol (4 ml) was mixed with 1 ml of methanolic solution having a final DPPH (Sigma) radical concentration of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm in a Hitachi U-2001 spectrophotometer. BHA, α-tocopherol and ascorbic acid were used as controls.

2.9. Chelating effects on ferrous ions

Chelating effect was determined according to the method of Dinis, Madeira, and Almeida (1994). The essential oil or its fractions (1–20 mg ml^{-1}) in methanol (1 ml) was mixed with 3.7 ml of methanol and 0.1 ml of 2 mM $FeCl_3 \bullet 4H_2O$ and the reaction was allowed to proceed for 30 s. After 0.1 ml of 5 mM ferrozine (Sigma) was added, the solution was mixed, left to stand for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm in a Hitachi U-2001 spectrophotometer. A lower absorbance indicates a higher chelating power. Ethylenediaminetetraacetic acid (EDTA) and citric acid were used as controls.

2.10. Statistical analysis

For the essential oil or its fractions, three samples were prepared for assays of every antioxidant attribute. The experimental data were subjected to an analysis of variance for a completely random design, as described by Steel, Torrie, and Dickey (1997) to determine the least significant difference at the level of 0.05.

Table 1
Fractions of the essential oil from the rhizome of *Curcuma zedoaria*

Fraction[a]	Solvent	Solvent ratio	Yield[b] (%)
F-1	Pentane	100	0.54e
F-2	Pentane/ether	75/25	83.7a
F-3	Pentane/ether	50/50	10.7b
F-4	Pentane/ether	25/75	1.89d
F-5	Ether	100	3.48c

[a] F-1, F-2, F-3, F-4, and F-5: Fractions of essential oil eluted from a silica gel-60 (55 g) column with various solvents (100 ml each).

[b] Means ($n = 3$) with different letters within a column are significantly different ($P < 0.05$).

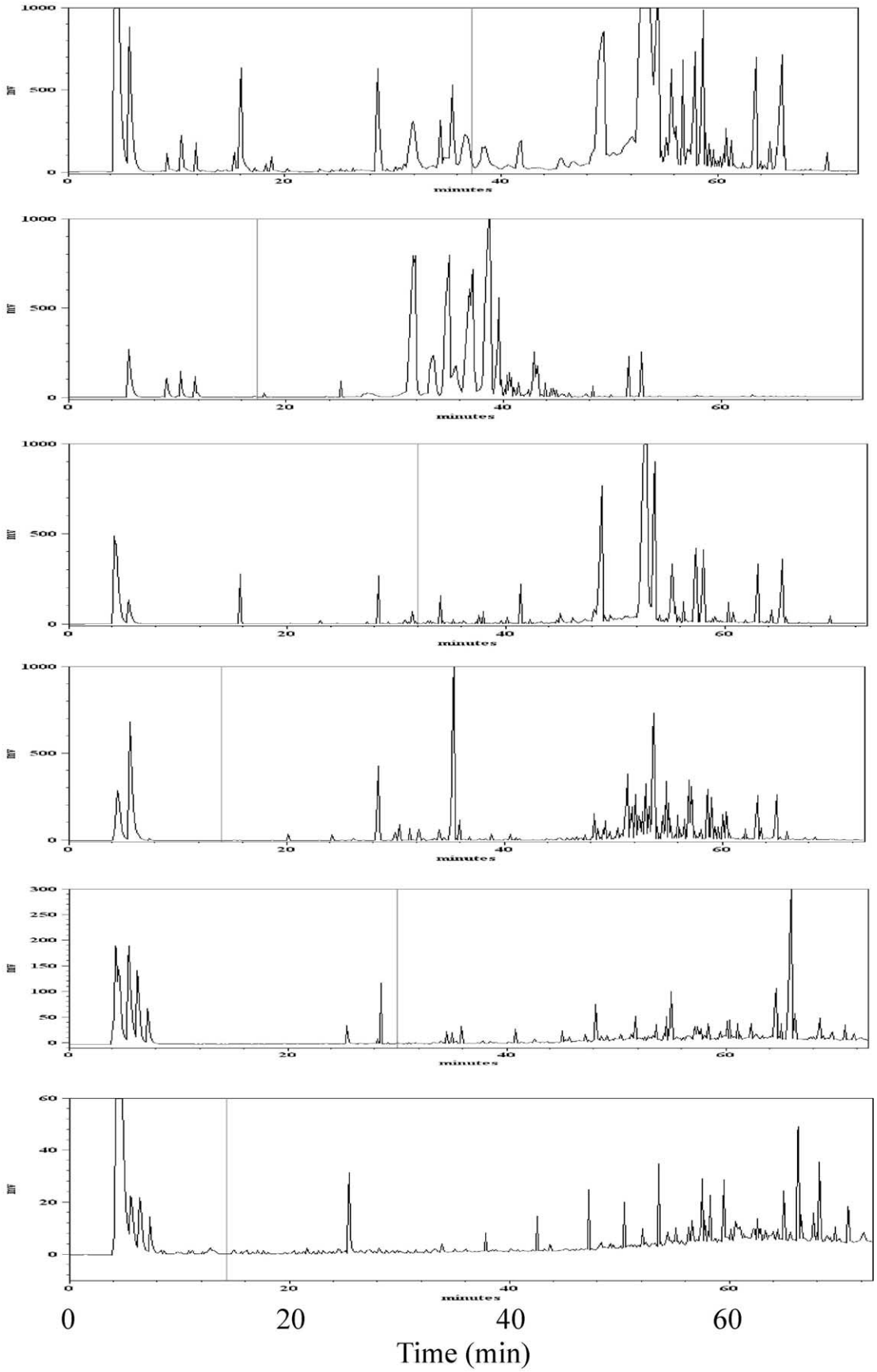

Fig. 1. Gas chromatograms of essential oil and its fractions (F-1, F-2, F-3, F-4 and F-5) from the rhizome of *Curcuma zedoaria* (from top to bottom).

3. Results and discussion

Using the simultaneous steam-distillation and solvent-extraction method, the essential oil content in the dried rhizome of *C. zedoaria* was determined to be only 0.63%, which was much lower than the water extract (7.86%) and methanolic extract (8.14%), and slightly lower than the supercritical carbon dioxide extracts (0.82% at 45 °C and 1500 psi for 2 h) from *C. zedoaria* rhizome (Chen, 2001). The essential oil was further separated in to 5 fractions by silica gel column chromatography. Among five fractions, the yields of Fractions 2 and 3 were 83.66 and 10.71%, respectively (Table 1).

The gas chromatographic profiles and compositions of the essential oil and its fractions were presented in Fig. 1 and Table 2, respectively. Totally, 36 compounds were identified in the essential oil, including 17 terpenes and 20 oxygen-containing compounds (13 alcohols and 6 ketones). These results are similar to the findings of Tang and Eisenbrand (1992) that the essential oil of *C. zedoaria* contained high ratios of terpene compounds in the identified compounds. Epicurzerenone, curzerene

Table 2
Composition of essential oil and its fractions from the rhizome of *Curcuma zedoaria*

Peak No.	Compound	Kovats index	Area[a] (%)					
			Total	F-1[b]	F-2[b]	F-3[b]	F-4[b]	F-5[b]
1	α-Pinene[c]	1018	0.36±0.01	1.10±0.13	–[d]	–	–	–
2	Camphene[c]	1062	0.59±0.02	1.30±0.11	–	–	–	–
3	β-Pinene[c]	1099	0.31±0.04	0.74±0.06	–	–	–	–
4	1,8-Cineole[c]	1219	1.99±0.03	–	2.07±0.32	–	–	–
5	2-Nonanone[c]	1388	0.74±0.02	–	–	5.14±1.21	–	–
6	2-Decanone[c]	1489	0.10±0.01	–	0.11±0.03	–	–	–
?	Camphor[c]	1516	1.72±0.07	0.07± <0.01	1.99±0.27	–	1.91±0.22	–
8	2-Undecanone[c]	1593	0.04± <0.01	0.05± <0.01	–	–	–	–
9	β-Elemene	1587	1.92±0.42	11.43±1.23	0.30±0.06	0.84±0.05	–	1.90±0.22
10	γ-Elemene	1636	0.20±0.06	0.33±0.41	0.08±0.01	0.50±0.07	0.33±0.02	–
11	β-Farnesene	1663	0.21±0.09	5.14±1.22	0.10±0.02	–	–	–
12	α-Humulene	1673	1.01±0.11	15.27±2.34	0.10±0.03	–	0.42±0.06	–
13	β-Himachalene	1684	0.45±0.02	0.81±0.22	0.12±0.04	–	0.49±0.11	–
14	Zingiberene[c]	1715	1.51±0.09	3.27±0.33	0.13±0.02	–	0.61±0.12	–
15	α-Terpineol[c]	1729	0.16±0.01	0.11±0.02	–	10.40±2.11	–	–
16	α-Selinene	1748	1.25±0.11	0.10±0.01	0.34±0.04	–	–	–
17	β-Selinene	1753	0.89±0.06	0.32±0.02	–	–	–	–
18	β-Bisabolene	1758	1.24±0.16	10.06±2.21	0.21±0.09	–	–	–
19	α-Curcumene	1769	1.03±0.31	17.95±0.24	0.32±0.02	–	0.20±0.01	–
20	Germacrene B	1827	1.17±0.23	5.32±0.31	0.18±0.03	–	0.53±0.14	–
21	α-Calacorene	1916	0.68±0.11	1.70±0.33	1.21±0.17	–	–	–
22	Calarene	1978	0.62±0.14	0.37±0.03	–	–	–	–
23	Curzerene	2076	10.36±1.45	–	10.66±2.23	–	0.33±0.07	0.33±0.11
24	β-Elemenone	2091	0.66±0.11	–	0.54±0.02	–	0.41±0.12	0.41±0.09
25	Epicurzerenone	2219	24.08±2.13	–	34.29±2.52	8.75±1.14	1.29±0.21	11.27±1.52
26	Curdione	2249	7.00±1.14	–	18.76±0.42	4.21±1.22	1.21±0.13	1.21±0.22
27	Elemol	2271	0.26±0.01	–	–	0.84±0.22	–	–
28	Curzereneone	2278	2.39±0.33	–	–	–	–	–
29	Spathulenol	2299	2.95±0.34	–	2.47±0.11	14.60±2.11	3.13±0.25	–
30	α-Cadinol	2315	0.45±0.11	–	0.91±0.07	4.99±1.12	0.42±0.17	–
31	Eudesmol	2352	1.43±0.41	–	–	14.97±2.14	–	–
32	Curcumol	2360	1.43±0.15	–	1.87±0.33	3.88±1.12	0.44±0.12	–
33	Isocurcumenol	2403	2.98±0.34	–	3.36±1.11	2.71±0.34	1.09±0.32	10.75±2.11
34	Farnesol	2405	0.31±0.12	–	0.12±0.02	3.17±0.44	1.52±0.25	–
35	Isospathulenol	2423	0.54±0.17	–	0.56±0.11	3.88±1.21	1.19±0.22	–
36	5-Isopropylidene-3,8-dimethyl-1(5H)-azulenone	>2500	4.30±0.46	–	4.89±0.34	–	20.19±2.41	3.80±1.10
Total			77.33±2.12	75.44±2.46	85.69±3.11	78.88±2.24	35.71±1.54	29.67±1.32

[a] Each value is expressed as means±S.D. ($n=3$).
[b] F-1, F-2, F-3, F-4, and F-5: Fractions of the essential oil eluted from a silica gel-60 (55 g) column with *n*-pentane, *n*-pentane-diethyl ether mixture (75/25, 50/50, 25/75, v/v), and diethyl ether (100 ml each), respectively.
[c] Mass spectra and Kovats indices are consistent with those of authentic compounds.
[d] Not detectable.

and curdione were found in the first, second and third highest amounts (24.1, 10.4 and 7.00%, respectively) in the essential oil. The major compounds in fraction 1 were β-elemene (11.5%), α-humulene (15.3%), β-bisabolene (10.1%), and α-curcumene (18.0%). In fraction 2, the major compounds were epicurzerenone (34.3%), curdione (18.8%), and curzerene (10.7%). In fraction 3, the major compounds were spathulenol (14.6%), eudesmol (15.0%), and α-terpineol (10.4%). In fraction 4, the major compounds were 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone (20.2%), and spathulenol (3.13%). In fraction 5, the major compounds were epicurzerenone (11.3%), and isocurcumenol (10.8%).

Epicurzerenone was high in the essential oil and found in fractions 2, 3 and 4 (Table 2). It was postulated that the elution of this compound was not completed by one single eluant, partially due to its high content and specific polarity. The contents of ketones were high in fraction 2 whereas fewer amounts of ketones and increased amounts of alcohols, such as curcumol and elemol, were found in fraction 3. Along with the increased polarity of the eluants, the eluted fractions consisted of more polar compounds. After fractionation using the silica gel column chromatography, minor components present in the essential oil could be enriched in the eluted fractions, and therefore, were easily recognized and identified.

Gas chromatographic profile and percent composition of the essential oil were similar to those of the supercritical carbon dioxide extracts from *C. zedoaria* (Chen, 2001). Only 31 compounds were found in the supercritical carbon dioxide extracts, excluding α-pinene, camphene, 2-nonanone, α-terpineol and 2-decanone. Similarly, epicurzerenone and curzerene were found in the first and second highest amounts (26.0–26.7 and 5.59–6.80%, respectively) in the supercritical carbon dioxide extracts (Chen, 2001). However, 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone was the third most abundant compound (Chen, 2001).

Using the DETBA method, the essential oil showed a high inhibition of peroxidation (76.3–87.1%) at 10 to 20 mg ml^{-1} (Fig. 2). Generally, the antioxidant activity of the essential oil steadily increased with the increased concentration, and was comparable to that of ascorbic acid and lower than that of BHA and α-tocopherol. The antioxidant activities of the essential oil and its fractions

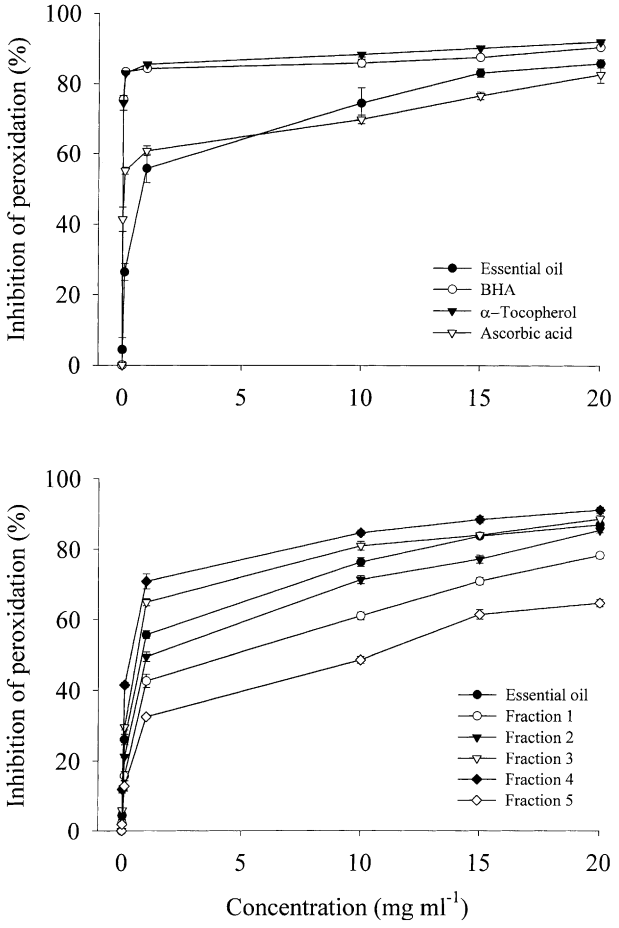

Fig. 2. Antioxidant activity of essential oil and its fractions from the rhizome of *Curcuma zedoaria* (1,3-diethyl-2-thiobarbituric acid method). Each value is expressed as mean ± standard deviation ($n = 3$).

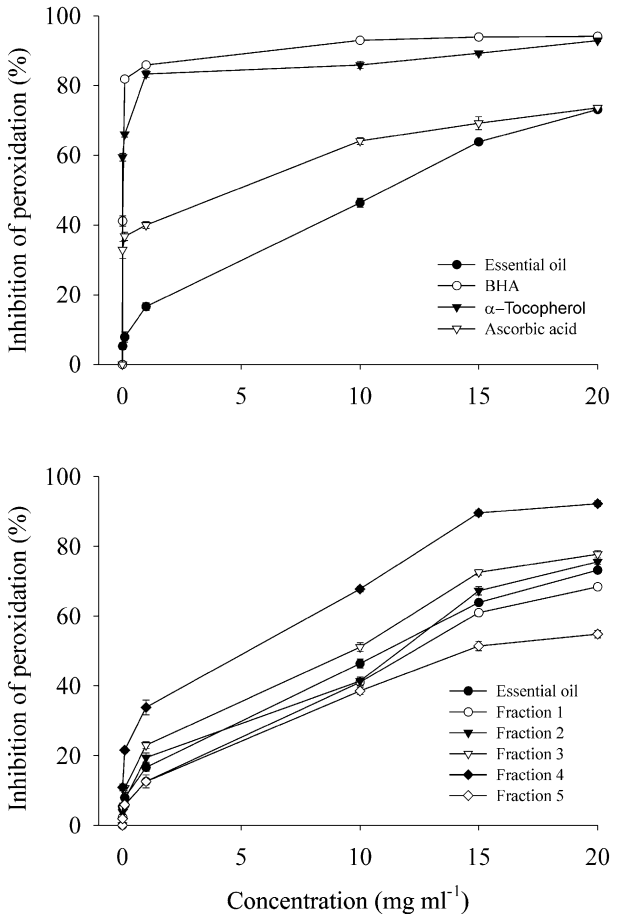

Fig. 3. Antioxidant activity of essential oil and its fractions from the rhizome of *Curcuma zedoaria* (thiobarbituric acid reactive substance method). Each value is expressed as mean ± standard deviation ($n = 3$).

followed the same trend and in the descending order of F-4 > F-3 > the essential oil > F-2 > F-1 > F-5 at 1–10 mg ml^{-1}. However, at 20 mg ml^{-1}, the antioxidant activities of the essential oil and fractions 2, 3 and 4 were similar (85.5–91.2%), and higher than those of fractions 1 and 5 (78.3 and 64.7%, respectively). At 20 mg ml^{-1}, the antioxidant activity of fraction 4 (91.2%) was comparable to those of BHA (90.8%) and α-tocopherol (92.3%).

Using the TBARS method, the essential oil showed a moderate inhibition of peroxidation (46.3–73.1%) at 10–20 mg ml^{-1} (Fig. 3). The antioxidant activity of the essential oil rapidly increased with the increased concentration, and was comparable to that of ascorbic acid at 20 mg ml^{-1} and much lower than those of BHA or α-tocopherol. The antioxidant activity of fraction 4 was the best among those of the essential oil and its fractions, and higher than that of ascorbic acid at 10–20 mg ml^{-1} and comparable to that of α-tocopherol at 15 mg ml^{-1} and those of BHA and α-tocopherol at 20 mg ml^{-1}. However, at 20 mg ml^{-1}, the antioxidant activities were in the descending order of F-4 (92.2%) > F-3≈F-2≈the essential oil (73.1–77.7%) > F-1 (68.3%) > F-5 (54.8%).

Using the conjugated diene method, the essential oil showed a moderate antioxidant activity (56.7–70.3%) at 10–20 mg ml^{-1} (Fig. 4). The antioxidant activity of fraction 2 was comparable to that of ascorbic acid at 10–20 mg ml^{-1}, whereas that of fraction 4 was comparable to that of ascorbic acid at 10–15 mg ml^{-1}. At 20 mg ml^{-1}, the antioxidant activity of fraction 4 (97.1%) was higher than that of ascorbic acid (84.7%) and α-tocopherol (92.7%), and similar to that of BHA (98.6%). However, at 20 mg ml^{-1}, the antioxidant activities were in the descending order of F-4 (97.1%) > F-2 (85.0%) > F-1≈F-3≈the essential oil (71.3–77.7%) > F-5 (51.3%).

Using three different methods to measure the antioxidant activities, two antioxidants, BHA and α-tocopherol, evidently consistently showed excellent antioxidant activities at concentrations as low as 0.1 mg ml^{-1}. Ascorbic acid showed a moderate antioxidant activity (40.2–61.5%) at 1 mg ml^{-1}. However, the essential oil from *C. zedoaria* showed a moderate to

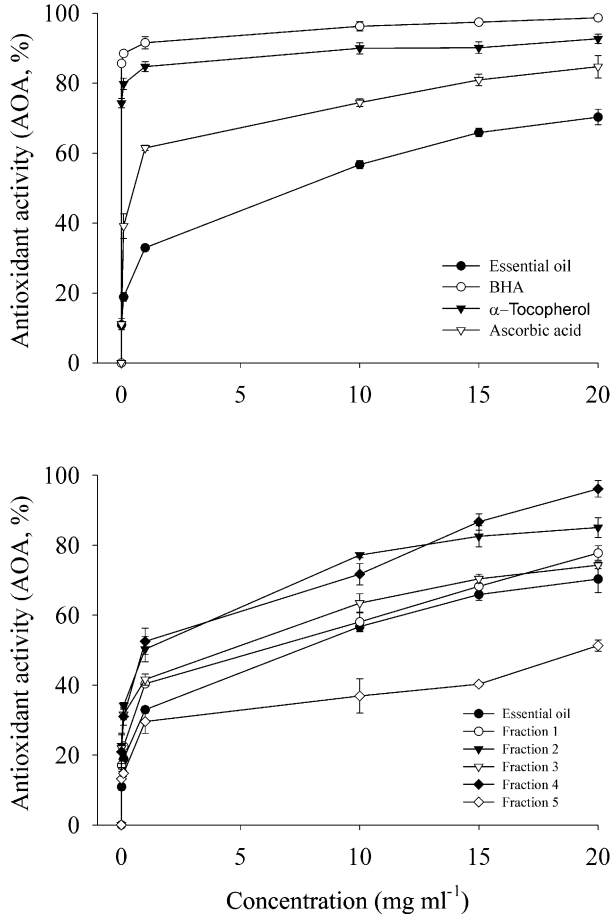

Fig. 4. Antioxidant activity of essential oil and its fractions from the rhizome of *Curcuma zedoaria* (conjugated diene method). Each value is expressed as mean ± standard deviation ($n = 3$).

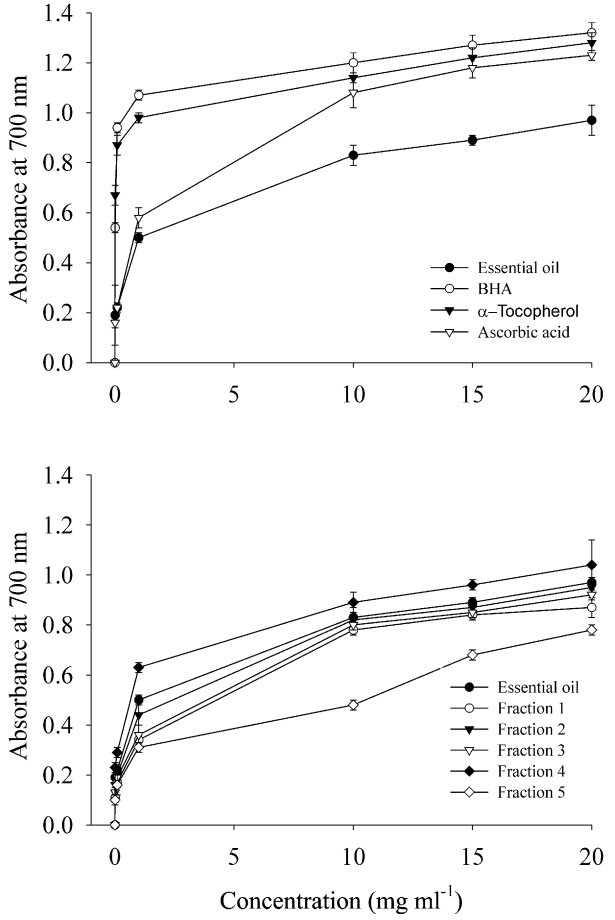

Fig. 5. Reducing power of essential oil and its fractions from the rhizome of *Curcuma zedoaria*. Each value is expressed as mean ± standard deviation ($n = 3$).

high antioxidant activity at 10–20 mg ml^{-1}. In addition, the antioxidant activities of the essential oil, in three methods tested, were all similar to those of the supercritical carbon dioxide extracts at 0.1–20 mg ml^{-1} (Chen, 2001). Among the fractions, fraction 4 exhibited the best antioxidant activity at 10–20 mg ml^{-1}. Obviously, fraction 4 contained some compounds effective in inhibiting lipid peroxidation. Nevertheless, fraction 4 only accounted for 1.89% of the essential oil (Table 1).

The reducing power of the essential oil increased with increased concentration (Fig. 5). However, the reducing power of the essential oil was much lower than those of ascorbic acid, BHA or α-tocopherol. At 20 mg ml^{-1}, the reducing powers were in the descending order of F-4 (1.04) > the essential oil ≈F-2≈F-3 (0.92–0.97) > F-5 (0.78). The reducing power of fraction 4 was the best among the essential oil and its fractions but still lower than those of ascorbic acid, BHA and α-tocopherol. Also, reducing powers of the essential oil (0.83–0.97) and fraction 4 (0.89–1.04) were lower than that of the supercritical carbon dioxide extracts at 10–20 mg ml^{-1} (1.05–1.18) (Chen, 2001).

The scavenging effect of the essential oil on DPPH radical linearly increased from 12.7 to 90.5% as concentrations increased from 0.1 to 15 mg ml^{-1} (Fig. 6). At 20 mg ml^{-1}, the scavenging effect of the essential oil was 96.8%, comparable to those of BHA (97.0%) and α-tocopherol (96.4%), and higher than that of ascorbic acid (91.0%). The scavenging effect of fraction 4 was higher than that of the essential oil at 1–10 mg ml^{-1}, but comparable to that of the essential oil at 15–20 mg ml^{-1}. However, the scavenging effects of BHA, ascorbic acid and α-tocopherol were much more effective at an extremely low concentration, and were 93.0, 87.6 and 38.0% at 0.01 mg ml^{-1}, respectively. At 20 mg ml^{-1}, scavenging effects were in the descending order of the essential oil ≈F-4 (96.8–97.4%) > F-3 (90.6%) > F-1≈F-2 (70.9–72.4%) > F-5 (52.7%). However, scavenging effects of the essential oil followed the same trend and were higher than those of the supercritical carbon dioxide extracts at 0.1–20 mg ml^{-1} (Chen, 2001).

The chelating effect of the essential oil on ferrous ion was 8.7% at 0.01 mg ml^{-1} and increased to be 23.2% at 20 mg ml^{-1}, slightly lower than that of citric acid (25.2%) (Fig. 7). However, the chelating ability of

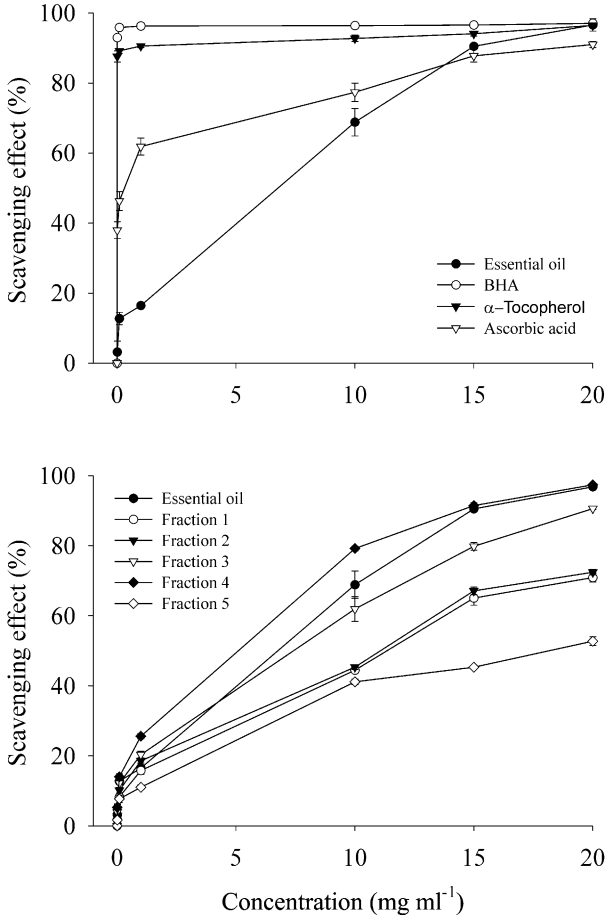

Fig. 6. Scavenging effect of essential oil and its fractions from the rhizome of *Curcuma zedoaria* on 1,1-diphenyl-2-picryl hydrazyl radical. Each value is expressed as mean±standard deviation ($n=3$).

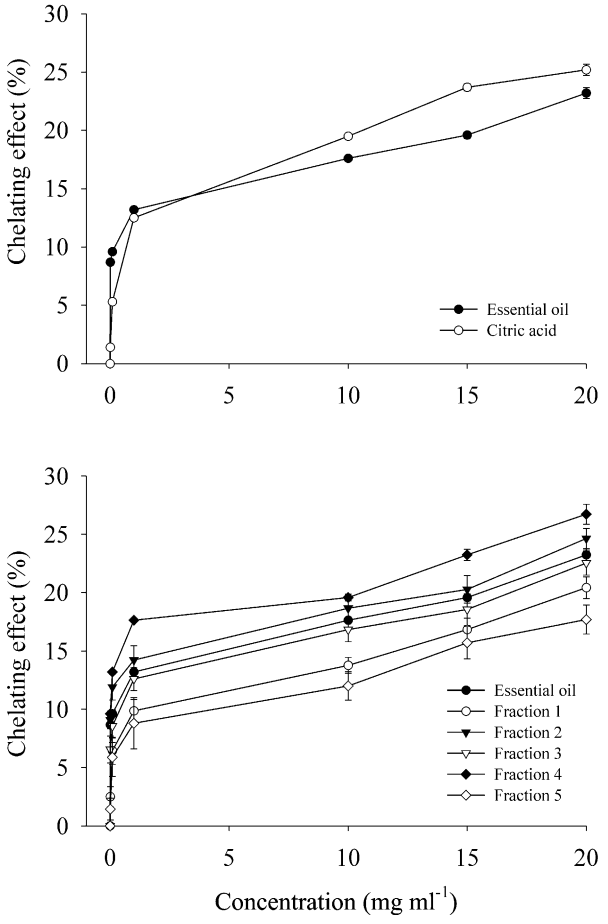

Fig. 7. Chelating effect of essential oil and its fractions from the rhizome of *Curcuma zedoaria* on ferrous ions. Each value is expressed as mean±standard deviation ($n=3$).

EDTA was 99.5% at 1 mg ml^{-1}. In addition, the chelating effects of the supercritical carbon dioxide extracts were low and in the range of 27.9–29.2% at 20 mg ml^{-1} (Chen, 2001). It was obvious that the chelating effect was not effective for the essential oil of *C. zedoaria*. The chelating effects of fractions 4 and 2 were consistently higher than those of the essential oil at 1–20 mg ml^{-1}. At 20 mg ml^{-1}, the chelating effects were in the descending order of F-4 (26.7%) > F-2 (24.6%) > F-3≈the essential oil (22.5–23.2%) > F-1 (20.4%) > F-5 (17.7%).

Ruberto and Baratta (2000) studied antioxidant activities of several essential oil components in two lipid model systems. In the conjugated diene method at 1000 ppm, α-pinene, β-pinene, camphene, α-terpineol, 1,8-cineole, camphor, α-humulene, 3-decanone and 2-undecanone showed a low inhibition of peroxidation (12.6, 27.6, 9.8, 28.1, 20.3, 6.6, 15.7, 0 and 11.0%, respectively) (Ruberto & Baratta, 2000), and only farnesol exhibited 46.7% inhibition of peroxidation. However, in the TBARS methods at 10^{-2} M, farnesol showed a low inhibition of peroxidation (16.6%) (Ruberto & Baratta, 2000). Evidently, these ten compounds were not effective antioxidants and might not be responsible for the antioxidant properties of the essential oil from *C. zedoaria* rhizome.

At 20 mg ml^{-1}, the essential oil of *C. zedoaria* was moderate to good in antioxidant activities, good in reducing power and excellent in scavenging effect on DPPH radical but low in chelating effect on ferrous ion. However, after fractionation, with regard to all antioxidant properties assayed, fraction 4 showed consistently better effects than the essential oil did. In addition, fractions 1 and 5 were less effective than the essential oil. Fractions 2 and 3, accounting for 94.4% of the essential oil, were similar to the essential oil in all antioxidant properties, except for scavenging effect on the DPPH radical. The major compounds in fraction 4 were 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone (20.2%), and spathulenol (3.13%). Since spathulenol was found to be 14.6% in fraction 3, the compound in fraction 4 responsible for better antioxidant properties might be 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone. To study the antioxidant mechanism by these compounds or some other potential compounds, further investigations are needed.

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