Composition and Antioxidant Properties of Essential Oils from *Curcuma* rhizome

SNU-YAO TSAI[1,*], SHIH-JENG HUANG[2], CHARNG-CHERNG CHYAU[3], CHING-HSUAN TSAI[4], CHU-CHUN WENG[4] AND JENG-LEUN MAU[4]
[1] *Department of Health and Nutrition Biotechnology, Asia University, Taiwan*
[2] *Department of Nutrition and Health Science, Chungchou Institute of Technology, Taiwan*
[3] *Department of Biotechnology, HungKuang University, Taiwan*
[4] *Department of Food Science and Biotechnology, National Chung-Hsing University, Taiwan*

ABSTRACT

Genus *Curcuma* (Zingiberaceae) has long been used as a folk medicine. The essential oils of dried rhizomes of *C. aromatica* Salisb., *C. longa* L. and *C. sichuanensis* X. X. were isolated using simultaneous steam-distillation and solvent-extraction apparatus. In total, twenty-three compounds were identified as essential oils, including ten terpenes, six alcohols, two ketones and five other compounds. The major compounds were curcumol (35.77%) and 1,8-cineole (12.22%) for *C. aromatica*, ar-turmerone (49.04%), humulene oxide (16.59%) and β-selinene (10.18%) for *C. longa*, and ar-turmerone (43.52%), β-selinene (13.36%) and δ-cadinene (13.22%) for *C. sichuanensis*. EC_{50} values of antioxidant activities of the essential oils were 3.19-19.63 mg/mL and the effectiveness was, in descending order: *C. longa* > *C. sichuanensis* > *C. aromatica*. EC_{50} values of scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl radicals were 8.29-15.54 mg/mL and the effectiveness was in a descending order: *C. aromatica* > C. *longa* > *C. sichuanensis*.

Key words: *Curcuma*, folk medicine, essential oil, antioxidant activity, scavenging ability.

1. INTRODUCTION

The genus *Curcuma* (Zingiberaceae) comprises more than seventy species of rhizomatous herb. Most of the *Curcuma* species grow in mountainous areas of the world, but some common species are often cultivated in gardens and used as a spice, food preservative and coloring agent and as medicinal plants. The volatile oils of the rhizomes and leaves of these plants can be prepared by steam distillation or solvent extraction, and show a wide spectrum of medicinal applications (Singh, G., Singh, O. P. & Maurya, 2002). The essential oil isolated from the rhizome of *C. zedoaria* has been characterized and shows antioxidant activities (Mau et al., 2003), antimicrobial activity and cytotoxicity (Lai et al., 2004).

In addition to *C. zedoaria*, three species of *Curcuma* are also currently available in Taiwan. *Curcuma aromatica* Salisb. has long been used as a medicinal plant for releasing pain, reducing inflammation, and as a flavouring spice in Indian and Taiwanese cuisine. It has been reported that the essential oil or extracts from the rhizome of *C. aromatica* also possessed anti-inflammatory activity (Jangde, Phadnaik & Bisen, 1998; Li, 1985), along with antitumor and pharmacological effects (Fu, 1984). The rhizome of *Curcuma longa* L., also known as *C. domestica* Val., is commonly used as a food spice, and contains insecticidal and antifungal

* Corresponding author. E-mail: sytsai@asia.edu.tw

agents (Singh et al., 2002). Chemical compositions of *C. aromatica* and *C. longa* have been studied (Zwaving & Bos, 1992; Jarikasem, Thubthimthed, Chawananoraseth & Suntorntanasat, 2005). However, the composition of the essential oil from another rhizome of *C. sichuanensis* X. X. is not available. Furthermore, the antioxidant properties of the essential oils from these three species have not been studied.

Accordingly, we extracted essential oils from the dried rhizomes of *C. aromatica*, *C. longa* and *C. sichuanensis* and identified the components. In addition, the antioxidant properties of the essential oils, including antioxidant activity and scavenging ability were determined.

2. MATERIALS AND METHODS

2.1 Plant Material

Dried rhizomes of *C. aromatica*, imported from Japan, and dried rhizomes of *C. longa* and *C. sichuanensis*, imported from Sichuan Province, the People's Republic of China, were all purchased from a traditional Chinese pharmacy in Changhua City, Taiwan. Samples were ground to 20 mesh in a comminuting mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany), and the ground sample (~1000 g) thus obtained was stored in the dark at 4 °C before use.

2.2 Isolation of Essential Oil

The essential oil was isolated by the simultaneous steam-distillation and solvent-extraction (SDE) method using Likens and Nickerson apparatus (Likens & Nickerson, 1964) with some modification by Filek, Bergamini and Lindner (1995). A Ground sample (100 g) was placed in the apparatus and deionised water (1000 mL) was added. A 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 SDE process was allowed to proceed for 2 h, and the extract thus obtained was dried over anhydrous sodium sulphate (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 until chemical analyses. The isolation of the essential oil was carried out in triplicate.

2.3 Chemical Characterization

Components in the essential oil 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 thicknesses, Chrompack, Middelburg, and 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. The column temperature was programmed

from 40 to 220 °C at 3 °C/min. Temperatures for GC injector and GC-MSD interface were 250 °C and 265 °C, respectively.

Components were identified on the basis of gas chromatographic retention indices, mass spectra from Wiley MS Chemstation Libraries (6th edition, G1034, Rev. C.00.00, Hewlett-Packard, Palo Alto, CA) and the literature (Adams, 1995). 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 a percentage of the peak area relative to total peak area. Kovats indices were calculated for each separate component against *n*-alkanes standard (C_8-C_{25}, Alltech Associates, Deerfield, IL) according to Schomberg and Dielmann (1973).

2.4 Antioxidant Activity

Antioxidant activity was determined by the conjugated diene method (Lingnert, Vallentin & Eriksson, 1979). The antioxidant activity assayed is the ability of the essential oil to inhibit the peroxidation of linoleic acid in which the double bond is converted to a conjugated diene. Each essential oil (0.01–20 mg/mL) in methanol (100 μL) was mixed with 2 mL of 10 mM linoleic acid (Sigma Chemical Co., St. Louis, MO, USA) to form an emulsion in 0.2 M sodium phosphate buffer (pH 6.6) 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 was added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer (Tokyo, Japan). The antioxidant activity was calculated as follows: Antioxidant activity (%) = [(ΔA234 of control – ΔA234 of sample)/ ΔA234 of control]×100.

2.5 Scavenging Ability on 1,1-Diphenyl-2-Picrylhydrazyl Radicals

Scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined according to the method of Shimada, Fujikawa, Yahara and Nakamura (1992). The scavenging ability assayed is the ability of the essential oil to react rapidly with DPPH radicals and reduce most DPPH radical molecules. Each essential oil (0.5–20 mg/mL) in methanol (4 mL) was mixed with 1 mL of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH (Sigma). The mixture was shaken vigorously and left to stand for 30 min. in the dark, and the absorbance was then measured at 517 nm against a blank. The scavenging ability was calculated as follows: Scavenging ability (%) = [(ΔA517 of control – ΔA517 of sample)/ ΔA517 of control]×100.

EC_{50} value (mg/mL) is the effective concentration at which the antioxidant activity was inhibited by 50% and DPPH radicals were scavenged by 50%, and was obtained by interpolation from linear regression analysis. Ascorbic acid, butylated hydroxyanisole (BHA), and α-tocopherol (all from Sigma) were used for comparison.

2.6 Statistical Analysis

For each species, the essential oil was prepared in triplicate for chemical

characterization and assays of every antioxidant attribute. The experimental data were subjected to an analysis of variance for a completely random design to determine the least significant difference at the level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Extraction Yields

Using the simultaneous SDE method, the essential oil contents in three dried rhizomes of *C. aromatica*, *C. longa* and *C. sichuanensis* were 7.11, 9.85 and 3.21 mg/g dry weight, respectively (Table 1). The essential oil content in the *C. zedoaria* was determined to be 6.30 mg/g for dried rhizomes (Mau et al., 2003) and 3.6 mg/g for fresh rhizomes (Purkayastha, Nath & Klinkby, 2006). In this research, *C. sichuanensis* had the lower essential oil content. Garg, Bansal, Gupta and Kumar (1999) found that the essential oil content of turmeric *C. longa* rhizomes was in the range of 0.16 to 1.94% fresh weight with an average of 0.71% fresh weight, whereas Raina et al. (2002) reported the essential oil of *C. longa* rhizomes to be 2.2% dry weight. It is thought that the cultivar and breeding location might be a reason for the difference in yields of essential oils.

3.2 Composition of Essential Oils

In total, twenty-three compounds were identified in the essential oils, including ten terpenes, six alcohols, two ketones and five other compounds. However, 4-terpineol and caryophyllene were not found in the essential oil of *C. sichuanensis*. These results are similar to the findings of Mau et al. (2003) in which the essential oil of *C. zedoaria* contained a lot of terpene compounds in the identified compounds. Generally, three essential oils showed similar profiles in chemical composition but different contents. The major compounds in the essential oils were curcumol (35.77%) and 1,8-cineole (12.22%) for *C. aromatica*, ar-turmerone (49.04%), humulene oxide (16.59%) and β-selinene (10.18%) for *C. longa*, and ar-turmerone (43.52%), β-selinene (13.36%) and δ-cadinene (13.22%) for *C. sichuanensis*.

Terpenes accounted for 16.06 - 35.78% of the three essential oils. Alcohols (51.58%) were predominantly present in the essential oil of *C. aromatica* whereas ketones were relatively higher in the essential oils of *C. longa* and *C. sichuanensis* (49.13 and 43.60%, respectively). The different contents in chemical classes might give rise to three essential oils with different characteristic flavor perceptions.

Zwaving and Bos (1992) first reported that the major compounds in the essential oil of *C. domestica* (*C. longa*) rhizome were ar-turmerone (24.7%), turmerone (29.5%) and turmerol (25.7%). Garg et al. (1999) noted that ar-turmerone (25.4%), α-turmerone (11.9%), β-pinene (9.2%) and β-turmerone (8.3%) were found in the essential oil of *C. longa* rhizome. In addition, Singh et al. (2002) found the major compounds in the essential oil of Indian *C. longa* rhizome to be ar-turmerone (51.8%) and ar-turmerol (11.9%). It seems that the essential oil

of *C. longa* rhizome from Indian and other regions contains ar-turmerone as its major component.

Table 1. *Composition of the essential oil of Curcuma rhizome*

Peak No	Compound	Kovats Index[2]	Peak area[1] (%)		
			C. aromatica	*C. longa*	*C. sichuanensis*
1	α-Pinene	1018	0.21 ± 0.10	0.14 ± 0.02	0.05 ± 0.01
2	β-Pinene	1099	0.06 ±< 0.01	0.24 ± 0.07	0.04 ± 0.01
3	Limonene	1212	0.06 ± 0.04	0.14 ± 0.03	0.03 ±< 0.01
4	1,8-Cineole	1224	12.22 ± 2.53	2.85 ± 0.75	0.09 ± 0.03
5	p-Cymene	1277	0.13 ± 0.03	0.82 ± 0.17	1.33 ± 0.49
6	2-Heptanol	1452	0.21 ± 0.05	0.06 ±< 0.01	0.03 ±< 0.01
7	Linalool oxide	1473	0.32 ± 0.03	0.06 ±< 0.01	0.08 ±< 0.01
8	2-Nonanol	1508	3.91 ± 0.15	0.04 ±< 0.01	0.11±< 0.01
9	Camphor	1516	0.05 ± 0.01	0.09 ± 0.01	0.08±< 0.01
10	Linalool	1533	6.36 ± 0.34	0.49 ± 0.05	0.05±< 0.01
11	β-Elemene	1587	4.43 ± 0.44	0.88 ± 0.12	3.17 ± 1.13
12	4-Terpineol	1606	0.67 ± 0.28	0.18 ± 0.04	Nd[3]
13	Caryophyllene	1634	2.05 ± 0.14	0.11 ± 0.04	nd
14	α-Humulene	1673	2.03 ± 0.56	3.41 ± 0.83	2.06 ± 0.65
15	α-Terpineol	1729	4.66 ± 0.11	2.90 ± 0.63	1.46 ± 0.16
16	α-Selinene	1748	2.30 ± 0.24	3.14 ± 0.53	2.52 ±< 0.01
17	β-Selinene	1753	2.85 ± 0.61	10.18 ± 0.46	13.36 ± 0.14
18	δ-Cadinene	1768	1.94 ± 0.27	0.74 ± 0.44	13.22 ± 1.24
19	Caryphyllene oxide	1990	5.87 ± 2.19	5.60 ± 0.40	3.25 ± 0.22
20	Humulene oxide	2038	6.11 ± 0.30	16.59 ± 1.16	7.95 ± 0.63
21	ar-Turmerone	2091	6.98 ± 0.35	49.04 ± 5.51	43.52 ± 3.17
22	Curcumol	2360	35.77 ± 2.27	1.45 ± 0.05	6.92 ± 0.53
23	Dodecanoic acid	> 2500	0.81 ± 0.05	0.85 ± 0.01	0.68 ± 0.04
Chemical class					
	Terpenes		16.06 ± 1.70B[4]	19.80 ± 4.58B	35.78 ± 4.49A
	Alcohols		51.58 ± 2.59A	5.12 ± 0.060B	8.57 ± 0.50B
	Ketones		7.03 ± 0.34B	49.13 ± 5.50A	43.60 ± 3.17A
	Others		25.33 ± 2.53A	25.95 ± 1.54A	12.05 ± 0.90B
Yield (mg/g)			7.11 ± 0.69A	9.85 ±1.89A	3.21 ± 0.09B

Notes. 1. Kovats retention indices calculated for CP-Wax 52B capillary column in GC-MSD.
2. Kovats retention indices calculated for CP-Wax 52B capillary column in GC-MSD.
3. Not detected.
4. Means with different letters within a row are significantly different ($P < 0.05$).

For the essential oil of *C. aromatica*, Zwaving and Bos (1992) characterized the major compounds as *ar*-curcumene (18.6%), β-curcumene (25.5%), xanthorrhizol (25.7%). Jarikasem et al. (2005) reported camphor (26.94%), ar-curcumene (23.18%) and xanthorrhizol (18.70%) to be the major compounds in the essential oil of *C. aromatica*. The discrepancy in major compounds in the essential oils might be due to the difference in cultivation regions.

1,8-Cineole in the essential oil may give rise to a fresh, diffusive, camphoraceous-cool odor (Arctander, 1969). β-Selinene gives a mild, woody,

warm, herbaceous-peppery odor whereas δ-cadinene gives a mild, dry-woody, slightly medicinal-tarry odor, similar to cumin and thyme (Arctander, 1969). In addition, humulene oxide gives a woody-spicy, dry and tenacious clove-like odor (Arctander,1969). However, the major compounds, ar-turmerone and curcumol, have the characteristic odor of turmeric and a slightly pungent bitter taste (Jain, Shrivastava, Nayak & Sumbhate, 2007).

3.3 Antioxidant Activity and Scavenging Ability

The results for antioxidant activity and scavenging ability on DPPH radicals of the three essential oils assayed herein are summarized in Table 2, and the EC_{50} values are calculated for comparison. Effectiveness of antioxidant activity and scavenging ability is inversely correlated with their EC_{50} values. With regard to the EC_{50} values of antioxidant activities by the conjugated diene method, the effectiveness of three essential oils was, in descending order: *C. longa* > *C. sichuanensis* > *C. aromatica*. However, EC_{50} values of antioxidant activities were 0.06, 0.07 and 2.66 mg/mL for BHA, α-tocopherol and ascorbic acid, respectively. Mau et al. (2003) found that EC_{50} value of antioxidant activity of the essential oil was 7.46 mg/mL for *C. zedoaria*. It seems that EC_{50} values of antioxidant activities of the essential oils were 3.19-19.63 mg/mL for *Curcuma* rhizomes. Lee, Weng and Mau (2007) found that EC_{50} values of antioxidant activities of ethanolic and hot water extracts from *C. aromatica* rhizome were 0.14 and 0.49 mg/mL, respectively. Obviously, these three essential oils were much less effective in antioxidant activity than ethanolic and hot water extracts from *Curcuma* rhizomes.

With regard to the EC_{50} values of scavenging abilities on DPPH radicals, the effectiveness of the three essential oils was in a descending order: *C. aromatica* > C. *longa* > *C. sichuanensis*. However, EC_{50} values of scavenging abilities were 0.05, 0.06 and 3.78 mg/mL for BHA, α-tocopherol and ascorbic acid, respectively. Mau et al. (2003) found that the EC_{50} value of the scavenging ability of the essential oil was 6.77 mg/mL for *C. zedoaria*. It seems that the essential oil of *C. zedoaria* is more effective in scavenging ability on DPPH radicals than that of three *Curcuma* rhizomes. Lee et al. (2007) found that the EC_{50} values of scavenging abilities of ethanolic and hot water extracts from *C. aromatica* rhizome were 0.27 and 2.76 mg/mL, respectively. Obviously, ethanolic and hot water extracts were much more effective in scavenging ability on DPPH radicals than the essential oils.

Singh et al. (2010) reported that essential oil of rhizomes (*Curcuma longa* Linn.) has higher antioxidants properties and ar-Turmerone and alpha-turmerone are the major constituents in essential oil. Ruberto and Baratta (2000) studied antioxidant activities of several essential oil components and showed that in the conjugated diene method at 1000 ppm (1 mg/mL), α-pinene, β-pinene, camphene, 1,8-cineole, α-terpineol, 3-decanone, camphor, α-humulene 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), and only farnesol exhibited 46.7% inhibition of peroxidation. However, these compounds alone or in synergy with other compounds present in the essential oil are responsible for the observed antioxidant activity and scavenging ability of *C. rhizome*.

Table 2. *EC_{50} values of the essential oil of Curcuma rhizome*

	EC_{50} Value[1] (mg/ml)	
	Antioxidant activity	Scavenging ability
C. aromatica	19.63 ± 0.93A[2]	8.29 ± 0.18C
C. longa	3.19 ± 0.15C	10.83 ± 0.16B
C. sichuanensis	3.79 ± 0.20B	15.54 ± 0.64A
Ascorbic acid	2.66 ± 0.12D	3.78 ± 0.17D
BHA	0.06 ± 0.01E	0.05 ± 0.01E
α-tocopherol	0.07 ± 0.01E	0.06 ± 0.01E

Notes. 1. the effective concentration at which the antioxidant activity was inhibited by 50%; and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50%. EC_{50} value was obtained by interpolation from linear regression analysis.
2. Each value is expressed as mean ± SD (n = 3). Means with different letters within a column are significantly different ($P < 0.05$).

4. CONCLUSIONS

Evidently, these ten compounds were not effective antioxidants and might not be responsible for the antioxidant properties of the three essential oils from *Curcuma* rhizomes. To study the antioxidant and scavenging mechanism of these major compounds found in the essential oils or some other potential compounds, further investigations are needed.

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Shu-Yao Tsai is currently an assistant professor in the Department of Health and Nutrition Biotechnology at Asia University in Taiwan. She received her Ph. D. degree from the Department of Food Science and Biotechnology at National Chung-Hsing University in 2006. Her main research interests include aroma chemicals and edible and medicinal mushrooms.

Shih-Jeng Huang is currently an assistant professor in the Department of Health and Nutrition Science at Chung-Chou Institute of Technology in Taiwan. He received his Ph.D. degree from the Department of Food Science at National Chung-Hsing University in 2005. His main research interests include aroma chemicals, fermentation, γ-irradiation technology and edible and medicinal mushrooms.

Charng-Cherng Chyau is currently a distinguished professor in the Department of Biotechnology from HungKuang University in Taiwan. He received his Master's degree from the Department of Food Science at National Chung-Hsing University. He has been a Research Fellow at the University of Birmingham in United Kingdom. His main research interests include separation technology, instrumental analysis, etc.

Ching-Hsuan Tsai is currently a research assistant in the Food and Drug Administration, Department of Health, Executive Yuan in Taiwan. She received her Master's degree from the Department of Food Science and Biotechnology at National Chung-Hsing University in 2010. Her main research interests include aroma chemicals and edible and medicinal mushrooms.

Chu-Chun Weng is currently working in the research and development department of Sally Biotech Co. Ltd., in Taiwan. She received her Master's degree from the Department of Food Science at National Chung-Hsing University in 2004. Her main research interests include development of functional foods.

Jeng-Leun Mau is currently a distinguished professor in the Department of Food Science and Biotechnology at National Chung-Hsing University in Taiwan. He received his Ph.D. degree from the Department of Food Science at The Pennsylvania State University in 1992. His main research interests include flavor chemistry and edible and medicinal mushrooms.