Communication

Thai Curcuma Species: Antioxidant and Bioactive Compounds

Supawadee Burapan 1, Mihyang Kim 2, Yingyong Paisooksantivatana 3, Bekir Engin Eser 4 and Jaehong Han 1,*

1 Metalloenzyme Research Group and Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Korea; i_chiu60@yahoo.com
2 Phytobean, AC. Ltd., Pori 2-gil 16-5, Gamcheon-myeon, Yechon 36810, Korea; mibcoterie@gmail.com
3 Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand; yp2624@yahoo.com
4 Department of Engineering, Aarhus University, Gustav Wieds Vej 10, 8000 Aarhus, Denmark; bekireser@eng.au.dk
* Correspondence: jaehongh@cau.ac.kr; Tel.: +82-31-670-4830

Received: 8 August 2020; Accepted: 1 September 2020; Published: 2 September 2020

Abstract: For the functional food applications, antioxidant properties and the bioactive compounds of the 23 Curcuma species commercially cultivated in Thailand were studied. Total phenolic content and DPPH radical scavenging activity were determined. The concentrations of eight bioactive compounds, including curcumin (1), demethoxycurcumin (2), bisdemethoxycurcumin (3), 1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (4), germacrone (5), furanodienone (6), zederone (7), and ar-turmerone (8), were determined from the Curcuma by HPLC. While the total phenolic content of C. longa was highest (22.3 ± 2.4 mg GAE/g, mg of gallic acid equivalents), C. Wan Na-Natong exhibited the highest DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity. Twenty-three Curcuma species showed characteristic distributions of the bioactive compounds, which can be utilized for the identification and authentication of the cultivated Curcuma species. C. longa contained the highest content of curcumin (1) (304.9 ± 0.1 mg/g) and C. angustifolia contained the highest content of germacrone (5) (373.9 ± 1.1 mg/g). It was noteworthy that 1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (4) was found only from C. comosa at a very high concentration (300.7 ± 1.4 mg/g). It was concluded that Thai Curcuma species have a great potential for the application of functional foods and ingredients.

Keywords: antioxidant; bioactive; Curcuma; curcuminoids; HPLC; sesquiterpenoids; total phenolic content

1. Introduction

Turmeric, Curcuma longa, is the only Curcuma species extensively cultivated and traded in the world [1]. Taxonomically, it belongs to the Curcuma genus of the Zingiberaceae family, and is mostly distributed in Asia [2,3]. Various beneficial health-promoting effects of C. longa, such as Alzheimer’s disease prevention, anti-inflammatory effects, and HIV-1 protease inhibition, were reported [4,5]. Curcuminoids and sesquiterpenoids were identified as the major bioactive constituents of C. longa [6]. More than a hundred species of Curcuma are reported worldwide, and about 30 species among them are cultivated and consumed in Thailand as food additives, cosmetics, and traditional medicines [7]. In particular, many of them are used for the treatment of various diseases due to the existence of bioactive compounds. However, bioactive compounds in other Curcuma species, other than C. longa, have never been studied extensively.

To expand the application of Curcuma species as functional foods, we have collected and cultivated 23 Thai Curcuma species widely consumed in Thailand. Total phenolic content and
antioxidant activity were measured in the ethanol extracts of dried *Curcuma* rhizomes. For the bioactive compound study, four curcuminoids and four sesquiterpenoids were isolated and used as reference compounds after complete characterizations, because these are popularly studied representative phytochemicals in *Curcuma* species. In detail, the curcuminoids curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin (3) were isolated from *C. longa*, and the other curcuminoid 1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (4) was isolated from *C. comosa*. The three germacrane-type sesquiterpenoids germacrone (5), furanodienone (6), and zederone (7) were isolated from *C. latifolia*, and ar-turmerone (8) was isolated from *C. zedoaria* (Figure 1). The compositions of these representative bioactive compounds in the *Curcuma* species were also analyzed by HPLC.

![Figure 1. Structure of curcuminoids and sesquiterpenoids isolated from *Curcuma* species.](image)

### 2. Materials and Methods

#### 2.1. Curcuma Species

The rhizomes of 23 *Curcuma* species were collected in Kanchanaburi in 2013 and cultivated in Tapong, Meaung Rayong, Thailand in 2014 throughout the year. The plants that formed flowers were processed to prepare depository specimens at Bangkok Herbarium, Plant Variety Protection Division, Department of Agriculture, Bangkok, Thailand.

#### 2.2. Plant Materials and Extraction

Fresh rhizomes of the 23 *Curcuma* species were rinsed several times with tap water to make them dust and debris free. The rhizomes were then cut into small pieces and dried at room temperature in the shade for 2–3 days. The dried samples were ground to a fine powder and kept in a deep freeze (−70 °C) for further experiments. For extraction, each sample (1 g) of *Curcuma* sp. was macerated in EtOH (20 mL) for 24 h. The ethanol extract was filtered and dried under reduced pressure. The residue was weighed for the extraction yield and dissolved in MeOH for the determination of total phenolic content and DPPH radical scavenging assay.

#### 2.3. Determination of Total Phenolic Contents

Total phenolic contents of all plant extracts were determined using Folin–Ciocalteu reagent as described by Singleton and Rossi [8]. The plant extracts were dissolved in MeOH (1 mL, 0.5 mg/mL). Samples were added to microtiter plates and mixed with 100 µL of a 10-fold diluted Folin–Ciocalteu reagent and 80 µL of 7.5% sodium carbonate. The absorbance at 765 nm was measured using microplate reader spectrophotometers (Spectramax190, Molecular Device, CA, USA) after 30 min. Total phenolic
The dried rhizomes (500 g) of *C. longa* were ground and extracted with EtOH (1 L) by maceration (24 h). The dried crude extracts (5 g) were suspended in water and extracted by EtOAc (500 mL × 3). The combined organic layer (500 mg) was isolated by vacuum liquid chromatography using hexanes and acetone.

Curcumin (1). Yellow powder; ESI m/z 369 [M + H]^+; m.p. 181–183 °C; Anal. calcd for C_{21}H_{20}O_{6}: C 70.99, H 5.36; found: C 69.72, H 5.51. \(^1\)H NMR (600 MHz, DMSO-d$_6$): δ 7.54 (2H, d, J = 15.8 Hz, H-4, 4′), 7.32 (2H, d, J = 2.0 Hz, H-6, 6′), 7.15 (2H, dd, J = 8.3 Hz, 2.0 Hz, H-10, 10′), 6.82 (2H, d, J = 8.1 Hz, H-9, 9′), 6.75 (2H, d, J = 15.8 Hz, H-3, 3′), 6.06 (1H, s, H-1), 3.84 (6H, s, 7-OCH$_3$, 7′-OCH$_3$). \(^1^3\)C NMR (151 MHz, DMSO-d$_6$): δ 183.26 (C-2, 2′), 183.43 (C-8, 8′), 149.03 (C-7, 7′), 140.76 (C-4, 4′), 126.37 (C-5, 5′), 123.21 (C-10, 10′), 121.13 (C-3, 3′), 115.76 (C-9, 9′), 111.35 (C-6, 6′), 100.91 (C-1), 55.76 (7-OCH$_3$, 7′-OCH$_3$).

Demethoxycurcumin (2). Yellow orange powder; ESI m/z 339 [M + H]^+; m.p. 170–171 °C; Anal. calcd for C_{20}H_{18}O$_5$: C 70.99, H 5.36; found: C 68.53, H 5.17. \(^1\)H NMR (600 MHz, DMSO-d$_6$): δ δ 10.0 (1H, br s, 8-OH), 7.56 (2H, d, J = 8.7 Hz, H-6, 6′), 7.57 (2H, dd, J = 15.8 Hz, 5.3 Hz, H-4, 4′), 7.31 (1H, d, J = 2.0 Hz, H-6′), 7.14 (1H, dd, J = 8.2 Hz, 2.0 Hz, H-10′), 6.82 (2H, dm, J = 7, 7′, 9, 9′), 6.75 (H, d, J = 16 Hz, H-3′), 6.69 (H, d, J = 16 Hz, H-3), 6.04 (1H, s, H-1), 3.51 (1H, s, H-1′), 3.83 (3H, s, 7-OCH$_3$). \(^1^3\)C NMR (125 MHz, DMSO-d$_6$): δ 183.26 (C-2, 2′), 183.56 (C-8, 8′), 160.25 (C-7, 7′), 148.43 (C-4, 4′), 141.13 (C-5′), 140.79 (C-4′), 130.76 (C-6, C-10), 126.76 (C-5′), 126.24 (C-5), 126.63 (C-10′), 121.46 (C-3′), 121.24 (C-3), 116.34 (C-7, C-9′), 111.68 (C-6′), 103.32 (C-1), 56.13 (OMe).

Bisdemethoxycurcumin (3). Yellow orange crystals; ESI m/z 309 [M + H]^+; m.p. 220–221 °C; Anal. calcd for C_{19}H_{16}O$_5$: C 74.01; H 5.23; found: C 69.72, H 5.51. \(^1\)H NMR (600 MHz, DMSO-d$_6$): δ 10.1 (2H, br s, 8,8′-OH), 7.57 (4H, dd, J = 6.6 Hz, 2.1 Hz, H-6, 6′, 10, 10′), 7.54 (2H, d, J = 16 Hz, H-4, 4′), 6.82 (4H, dm, J = 8.6 Hz, 7′, 9, 9′), 6.70 (2H, d, J = 16 Hz, H-3, 3′), 6.04 (1H, s, H-1), 3.51 (1H, s, H-1′). \(^1^3\)C NMR (151 MHz, DMSO-d$_6$): δ 183.32 (C-2, 2′), 159.88 (C-8, 8′), 140.48 (C-4, 4′), 130.44 (C-6, 6′, 10, 10′), 125.93 (C-5, 5′), 120.90 (C-3, 3′), 116.04 (C-7, 7′, 9, 9′), 101.05 (C-1).

The dried rhizome (100 g) of *C. comosa* was ground and macerated with MeOH (200 mL) for 24 h. The dried crude extracts (2 g) were partitioned by EtOAc and water (300 mL × 3). The EtOAc extract (300 mg) was further isolated by vacuum liquid chromatography using hexanes and acetone. Fractions 12–15 gave 4 (48 mg). 1,7-Diphenyl-(4E,6E)-4,6-heptadien-3-ol (4) Pale yellow solid; ESI m/z 247 [M + H]^+; Anal. calcd for C$_{19}$H$_{18}$O: C 86.99, H 6.92; found: C 86.37, H 7.59. \(^1\)H NMR (300 MHz, CDCl$_3$): δ 7.27 (2H, br, H-3′, H-5′), 6.75 (2H), 5.83 (H-4), 4.21 (1H, s, OH), 2.71 (H-7).
The sesquiterpenoids were isolated from the ethanol extract of *C. latifolia* by the same procedure as for curcuminoinds. Germacrone (5) was isolated from fractions 9–10 (18 mg), furanodienone (6) was isolated from fraction 15 (10 mg), and zederone (7) was isolated from fractions 20–22 (38 mg).

Germacrone (5). White crystal; EI m/z 218 [M]+; m.p. 55–56 °C; Anal. calcld for C_{15}H_{22}O: C 82.52, H 10.16, found: C 80.81, H 9.83. 

H NMR (600 MHz, CDCl3): δ 4.99 (1H, br d, J = 12.2 Hz, H-1), 4.71 (1H, br d, J = 11.2 Hz, H-5), 3.41 (1H, d, J = 10.6 Hz, H-9a), 2.99–2.83 (3H, m, H-6a, H-6b, H-9b), 2.37 (1H, m, H-2a), 2.19–2.05 (3H, m, H-2b, H-3a, H-3b), 1.78 (3H, s, 13-CH3), 1.73 (3H, s, 12-CH3), 1.63 (3H, s, 14-CH3), 1.44 (3H, s, 15-CH3). 

C NMR (151 MHz, CDCl3): δ 189.81 (C-6), 156.52 (C-8), 145.79 (C-4), 135.39 (C-10), 132.68 (C-5), 129.48 (C-7), 126.68 (C-10), 125.38 (C-5), 55.91 (C-9), 38.09 (C-3), 29.23 (C-6), 24.09 (C-2), 22.34 (C-13), 19.90 (C-12), 16.72 (C-14), 15.59 (C-15).

Furanodienone (6). Yellow pale oil; EI m/z 230 [M]+; m.p. 84–86 °C; Anal. calcld for C_{15}H_{18}O_{2}: C 78.23, H 7.88, found: C 64.84, H 6.91. 

H NMR (600 MHz, CDCl3): δ 7.08 (1H, m, H-12), 5.81 (1H, m, H-5), 5.18 (1H, br dd, J = 11.7 Hz, 4.3 Hz, H-1), 3.70 (2H, br d, J = 22 Hz, H-9), 2.47 (1H, dt, J = 11.4 Hz, 3.6 Hz, H-3a), 2.32 (1H, m, H-2a), 2.18 (1H, m, H-2b), 2.13 (3H, d, J = 1.2 Hz, 13-CH3), 2.00 (3H, d, J = 1.2 Hz, 15-CH3), 1.89 (1H, m, H-3b), 1.31 (3H, bd, J = 0.7 Hz, 14-CH3). 

C NMR (151 MHz, CDCl3): δ 189.81 (C-6), 156.52 (C-8), 145.79 (C-4), 135.39 (C-10), 132.44 (C-5), 130.51 (C-1), 123.71 (C-11), 122.18 (C-7), 41.70 (C-3), 40.66 (C-9), 26.44 (C-2), 18.98 (C-15), 15.78 (C-14), 9.55 (C-13).

Zederone (7). White crystal; EI m/z 246 [M]+; m.p. 149–151 °C; (69.13 %C, 7.11 %H) Anal. calcld for C_{15}H_{18}O_{2}: C 73.15, H 7.37, found: C 69.13, H 7.11. 

H NMR (600 MHz, CDCl3): δ 7.08 (1H, m, H-12), 5.48 (1H, m, H-1), 3.81 (1H, s, H-5), 3.75 (1H, d, J = 16.3 Hz, H-9a), 3.69 (1H, d, J = 16.3 Hz, H-9b), 2.52 (1H, m, H-2a), 2.30 (1H, dt, J = 13.1 Hz, 3.5 Hz, H-3a), 2.26–2.20 (1H, m, H-2b), 2.11 (3H, d, J = 1.3 Hz, 13-CH3), 1.34 (3H, d, J = 0.8 Hz, 15-CH3), 1.32–1.25 (1H, m, H-3b). 

C NMR (151 MHz, CDCl3): δ 192.19 (C-6), 157.08 (C-8), 138.05 (C-12), 131.19 (C-1), 131.04 (C-10), 123.24 (C-11), 122.21 (C-7), 66.54 (C-5), 63.96 (C-4), 41.88 (C-9), 37.98 (C-3), 24.64 (C-2), 15.72 (C-14), 15.14 (C-15), 10.26 (C-13).

*ar*-Turmerone (8) was isolated from *C. zedoaira*, and 65 mg of 8 was obtained from 100 g of the dried rhizomes. *ar*-Turmerone (8). Colorless oil; EI m/z 216 [M]+; Anal. calcld for C_{15}H_{20}O: C 83.28, H 9.32, found: C 79.31, H 8.82. 

H NMR (600 MHz, CDCl3): δ 7.10 (4H, aromatic, H), 6.02 (1H, J = 1.3 Hz), 3.28 (1H, dd, J = 8.1 Hz), 2.70 (1H, dd, J = 15.6 Hz), 2.60 (1H, dd, J = 15.6 Hz), 2.31 (3H, s, -CH3), 2.10 (3H, d, J = 1.3 Hz, -CH3), 1.85 (3H, d, J = 1.3 Hz, -CH3), 1.25 (3H, d, J = 1.3 Hz, 12-CH3). 

C NMR (151 MHz, CDCl3): δ 199.85, 155.06, 143.68, 135.53, 129.10, 126.65, 124.08, 52.68, 35.28, 27.63, 21.98, 20.97, 20.70.

2.6. Validation of HPLC Analysis

Validation of HPLC analysis was performed using the purified reference compounds. Calibration curves were constructed from the HPLC peak areas of the reference standards, 1–8, versus their concentrations. Linearity was calculated by measuring the eight points of the calibration curve in the range of 0.003125-0.4 mM (1–3) and 0.00625-0.8 mM (4–8) with triplicate measurements. The limits of detection (LOD) for 1–8 were found at the micromolar level. Demethoxycurcumin (2) and furanodienone (6) showed the lowest LOD (2.0 μM) and limits of quantification (LOQ) at 420 nm and 245 nm, respectively (Table 1).
Table 1. Validation of HPLC assay of curcuminoids and sesquiterpenoids.

| Compounds                        | Accuracy 1 | Linearity | Correlation Coefficient ($R^2$) | LOD (mM) | LOQ (mM) |
|----------------------------------|------------|-----------|---------------------------------|----------|----------|
| Curcumin (1)                     | 104.8 ± 7.9| $y = 56,277,482x + 476,424$ | 0.9951 | 0.0081 | 0.0245 |
| Demethoxycurcumin (2)            | 101.0 ± 7.9| $y = 50,455,350x + 56,885$  | 0.9997 | 0.0200 | 0.0061 |
| Bis-demethoxycurcumin (3)        | 96.5 ± 16.2| $y = 54,929,237x + 369,859$ | 0.9964 | 0.0069 | 0.0209 |
| 1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (4) | 106.9 ± 11.6| $y = 28,237,427x + 612,834$ | 0.9963 | 0.0125 | 0.0077 |
| Germacrone (5)                   | 97.5 ± 16.6| $y = 3,051,741x + 22,600$  | 0.9977 | 0.0110 | 0.0333 |
| Furanodienone (6)                | 101.9 ± 6.8| $y = 8,043,402x − 10,111$ | 0.9999 | 0.0020 | 0.0060 |
| Zederone (7)                     | 99.9 ± 5.1 | $y = 5,130,378x + 25,981$  | 0.9999 | 0.0026 | 0.0080 |
| α-Turmerone (8)                  | 98.5 ± 15.0| $y = 35,723,577x + 423,877$ | 0.9962 | 0.0125 | 0.0379 |

1 All values are presented as the mean ± SD of triplicate determinations.

2.7. Compositional Analysis of Curcuma Species

Each sample (1 g) of the Curcuma species was macerated in EtOH (20 mL) for 24 h. The extract was filtered and dried under reduced pressure. The dried residue was dissolved in DMF (N,N-dimethylformamide, 1 mg/1 mL) for the analysis with a Finnigan Surveyor Plus HPLC system. The mobile phase comprised 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in MeCN (solvent B). The injection volume for HPLC analysis was 10 µL and the flow was 1.0 mL/min. For the quantitative analysis, UV absorption at 420 nm was adopted for 1–3, and UV absorption at 245 nm was adopted for 4–8.

2.8. Statistical Analyses

All measurements were performed in triplicate. Data were processed by Microsoft Excel and reported as means ± standard deviation.

3. Results and Discussion

3.1. Taxonomy of Curcuma Species

Among the 23 Curcuma species, 11 Curcuma species, such as C. manga, C. aeruginosa, C. comosa, C. aurantiaca, C. aromatic, C. latifolia, C. zedoaria, C. longa, C. parviflora, C. angustifolia, and C. petiolate, were identified by the taxonomist at the Bangkok Herbarium. The other 12 species are new species, and designated by common names in this report (Table 2).

Table 2. Total phenolic content and antioxidant activity of Curcuma species.

| Curcuma Species          | Extraction Yield (%) | Total Phenolic Content (mg GAE/g Dry Weight) | Antioxidant Activity (%) 1 |
|--------------------------|----------------------|---------------------------------------------|---------------------------|
| Curcuma Wan Ma-Leung     | 6.5 ± 0.4            | 0.9 ± 0.0                                   | 18.2 ± 0.3                |
| Curcumamangga            | 6.9 ± 0.3            | 0.6 ± 0.3                                   | 9.6 ± 0.5                 |
| Curcuma Wan Ma-Hor       | 8.9 ± 0.2            | 0.5 ± 0.1                                   | 13.2 ± 0.5                |
| Curcuma Wan Khamin-Dam   | 13.5 ± 0.2           | 3.9 ± 0.4                                   | 20.4 ± 0.4                |
| Curcuma Wan Rang- Jud    | 6.3 ± 0.3            | 0.4 ± 0.1                                   | 11.2 ± 0.2                |
| Curcumaaeruginosa        | 8.6 ± 0.2            | 3.2 ± 0.3                                   | 21.5 ± 0.3                |
Table 2. Cont.

| Curcuma Species                  | Extraction Yield (%) | Total Phenolic Content (mg GAE/g Dry Weight) | Antioxidant Activity (%) ¹ |
|----------------------------------|----------------------|---------------------------------------------|---------------------------|
| Curcuma comosa                   | 16.8 ± 0.3           | 4.2 ± 0.1                                   | 90.0 ± 0.3                |
| Curcuma Wan Kanta-Mala           | 12.0 ± 1.0           | 4.8 ± 0.1                                   | 12.7 ± 0.3                |
| Curcumaaurantiaca                | 11.9 ± 0.1           | 1.5 ± 0.1                                   | 15.2 ± 0.4                |
| Curcumaaromatica                 | 18.5 ± 0.3           | 11.0 ± 0.2                                  | 20.8 ± 0.1                |
| Curcuma latifolia                | 20.3 ± 0.2           | 12.9 ± 0.3                                  | 58.6 ± 0.0                |
| Curcumazedoaria                  | 8.5 ± 0.6            | 9.3 ± 0.7                                   | 65.7 ± 0.2                |
| Curcumalonga                     | 21.6 ± 0.5           | 22.3 ± 2.4                                  | 73.9 ± 0.1                |
| Curcumaparviflora                | 11.4 ± 0.5           | 15.3 ± 1.2                                  | 17.5 ± 0.5                |
| Curcumaangustifolia              | 3.2 ± 0.3            | 2.6 ± 0.3                                   | 3.0 ± 0.2                 |
| Curcuma Wan Khabitong            | 11.7 ± 0.1           | 4.6 ± 0.1                                   | 17.0 ± 0.1                |
| Curcuma Wan Pataba               | 5.8 ± 0.2            | 3.3 ± 0.1                                   | 44.5 ± 0.8                |
| Curcuma Wan Kortong              | 8.4 ± 0.5            | 3.3 ± 0.1                                   | 10.4 ± 0.4                |
| Curcuma Wan Na-Natong            | 3.7 ± 0.2            | 5.0 ± 0.1                                   | 91.8 ± 0.6                |
| Curcumapeiolata                  | 5.5 ± 0.1            | 2.8 ± 0.4                                   | 11.8 ± 0.4                |
| Curcuma Wan Khamin-Khao-Padtalod| 6.2 ± 0.3            | 1.9 ± 0.8                                   | 7.7 ± 0.2                 |
| Curcuma Wan Chai-Dam             | 3.9 ± 0.2            | 8.9 ± 0.2                                   | 89.8 ± 0.6                |
| Curcuma Wan Khamintong           | 8.6 ± 0.2            | 7.7 ± 1.3                                   | 33.3 ± 0.7                |

¹ Antioxidant activity (%): final concentration of sample = 100 µg/mL and ascorbic acid final concentration = 10 µg/mL (95.1 ± 0.2).

3.2. Total Phenolic Content and Antioxidant Activity

Total phenolic contents of the Curcuma species varied from 0.4 ± 0.1 to 22.3 ± 2.4 mg GAE/g. The rhizomes of C. longa contained the highest phenolic contents (22.3 ± 2.4 mg GAE/g), followed by C. parfivlora (15.3 ± 1.2 mg GAE/g) and C. latifolia (12.9 ± 0.3 mg GAE/g). The DPPH radical scavenging activity of Curcuma extracts is also presented in Table 2. Curcuma Wan Na-Natong showed the highest DPPH radical scavenging activity (91.8 ± 0.6%), followed by C. comosa (90.0 ± 0.3%) and Curcuma Wan Chai-Dam (89.8 ± 0.6%). These three species showed very strong antioxidant activity, even stronger than C. longa, and require further study.

From the results, it was clear that total phenolic contents of Curcuma extracts are not necessarily correlated with the antioxidant activity (Figure 2). Among the three most antioxidant Curcuma rhizomes, C. Wan Na-Natong and C. Wan Chai-Dam are the new species that have never been extensively studied, regardless of their local popularity. The correlation chart (Figure 2) shows that the total phenolic content and DPPH radical scavenging antioxidant activity among the Curcuma species are not necessarily linearly correlated. This is believed to be due to different phytochemical compositions of Curcuma species, as discussed below.
3.3. Isolation of Bioactive Curcuminoids and Sesquiterpenoids

Curcuminoids 1–3 were isolated from C. longa, and 4 was isolated from C. comosa. Sesquiterpenoids 5–7 were isolated from C. latifolia for the first time in this study. Compound 8 was isolated from C. zedoaria. The structures of the isolated reference compounds (Figure 1) were confirmed by the comparison of melting point, elemental analysis, $^1$H, and $^{13}$C NMR data (see the Supplementary Materials) [9–16].

3.4. Chemical Composition of 23 Curcuma Species

Curcuminoids 1–3 are considered as the most characteristic bioactive secondary metabolites found in turmeric. Therefore, we analyzed the collected Curcuma species to find out whether the rhizomes contain 1–3. The EtOH extracts (1 mg/1 mL) were analyzed with HPLC at 420 nm [17,18], and only ten species were found to contain curcuminoids (Table 3). Furthermore, only three Curcuma species, C. zedoaria, C. longa, and Curcuma Wan Khamintong, contained all three curcuminoids (Figure 3). Trace amounts of curcuminoids were identified from C. manga, Curcuma Wan Rang-Jud, C. comosa, C. latifolia, C. parviflora, Curcuma Wan Pataba, Curcuma Wan Na-Natong, and C. petiolate under LOQ, only when the higher concentrations of the analytes were analyzed by HPLC. It is noticeable that C. longa was found to contain the highest curcuminoid content (653 mg/g). Even though curcumin (1) is the representative bioactive compound of turmeric, only nine Curcuma species contained curcumin (1). Demethoxycurcumin (2) was the major curcuminoid of four other Curcuma species, C. aeruginosa, C. aurantiaca, C. aromatica, and C. zedoaria.
Table 3. Analysis of curcuminoids from the Curcuma species.

| Curcuma Species              | 1   | 2   | 3    |
|-----------------------------|-----|-----|------|
| Curcuma Wan Ma-Leung        | 5.2 ± 0.1 | 4.1 ± 0.0 | ND 1 |
| Curcuma Wan Ma-Hor          | ND  | 0.2 ± 0.0 | ND   |
| Curcuma aeruginosa          | 35.5 ± 0.7 | 107.2 ± 1.0 | ND   |
| Curcuma aurantiaca          | 3.2 ± 0.0  | 6.5 ± 0.0  | ND   |
| Curcuma aromatica           | 24.3 ± 0.1 | 112.5 ± 1.0 | ND   |
| Curcuma zedoaria            | 72.3 ± 0.6  | 201.5 ± 1.5  | 28.2 ± 0.8 |
| Curcuma longa               | 304.9 ± 0.1 | 189.2 ± 0.4  | 158.8 ± 0.7 |
| Curcuma Wan Khabitong       | 10.2 ± 0.1  | 6.6 ± 0.0   | ND   |
| Curcuma Wan Kortong         | 0.1 ± 0.0   | 1.8 ± 0.0   | ND   |
| Curcuma Wan Khamintong      | 47.2 ± 0.1  | 37.1 ± 0.1  | 8.0 ± 0.0   |

1 ND = in trace below detection limit.

![HPLC chromatogram](image-url)

**Figure 3.** HPLC chromatogram (420 nm) for C. zedoaria, C. longa, and Curcuma Wan Khamintong; I: curcumin (1), II: demethoxycurcumin (2), III: bisdemethoxycurcumin (3).

From Indonesian Curcuma species, C. mangga, C. heyneana, C. aeruginosa, and C. soloensis were reported to contain curcuminoids [19]. Our analysis also confirmed that C. mangga and C. aeruginosa contained curcuminoids. C. aurantiaca contained high contents of 1–3, and this is the first report of the identification of 1–3 in the rhizomes of C. aurantiaca. Diarylheptanoid 1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol (4) belongs to the curcuminoids, but it was found only from C. comosa in a high content of 300.7 ± 1.4 mg/g. It is noteworthy that C. comosa does not contain 1–3.
There are more than 100 different sesquiterpenoids reported from Curcuma rhizomes [20], and these secondary bioactive metabolites are also responsible for some of the biological activities observed from the rhizomes of Curcuma species. Therefore, we isolated three germacrane-type sesquiterpenoids, germacrone (5), furanodienone (6), and zederone (7), which is the first report from C. latifolia. In addition, ar-turmerone (8) was isolated from C. zedoaria for the first time. Germacrone (5) is known to exhibit anti-inflammatory [21], antiviral [22], and anti-cancer activities [23], and was found in the various Curcuma species, such as C. aromatica, C. amanda, C. aromatic, C. xanthrhiza, and C. zedoaria.

Among the 23 Curcuma species analyzed, 21 species were found to contain at least one of the four sesquiterpenoids, and only two Curcuma species, C. aurantiaca and C. zedoaria, contained all four sesquiterpenoids (Table 4). Generally, germacrone (5) was the most abundant sesquiterpenoid in the Curcuma species, and C. angustifolia contained only 5, out of the eight reference compounds. In particular, C. angustifolia showed a very high concentration of 5 (37% of extract), and Curcuma Wan Khamin-Khao-Padtalod contained only ar-turmerone (8).

| Curcuma Species                      | Sesquiterpenoids (mg/g) | 5   | 6   | 7   | 8   |
|-------------------------------------|-------------------------|-----|-----|-----|-----|
| Curcuma Wan Ma-Leung                | 42.6 ± 0.9              | ND  | ND  | ND  | ND  |
| Curcuma Wan Ma-Hor                  | 47.5 ± 0.7              | 4.0 ± 0.0 | 6.6 ± 0.2 | ND  | ND  |
| Curcuma Wan Khamin-Dam              | 126.5 ± 0.3             | 18.5 ± 0.1 | ND  | ND  | ND  |
| Curcuma aeruginosa                  | ND                      | 3.1 ± 0.0 | ND  | ND  | ND  |
| Curcuma comosa                      | 31.1 ± 0.2              | 81.0 ± 0.9 | 37.6 ± 0.2 | ND  | ND  |
| Curcuma Wan Kanta-Mala              | 106.9 ± 0.4             | 15.7 ± 0.1 | ND  | ND  | ND  |
| Curcuma aurantiaca                  | 36.5 ± 0.4              | 15.4 ± 0.5 | 8.6 ± 0.1 | 4.9 ± 0.0 | ND  |
| Curcuma aromatic                    | 73.7 ± 2.8              | 4.7 ± 0.0 | 33.2 ± 0.6 | ND  | ND  |
| Curcuma latifolia                   | 56.2 ± 0.3              | 73.3 ± 1.1 | 61.3 ± 0.1 | ND  | ND  |
| Curcuma zedoaria                    | 7.5 ± 0.1               | 15.5 ± 0.4 | 3.3 ± 0.0 | 53.9 ± 0.2 | ND  |
| Curcuma longa                       | ND                      | ND  | ND  | 78.4 ± 0.2 | ND  |
| Curcuma parviflora                  | ND                      | 3.6 ± 0.1 | ND  | ND  | ND  |
| Curcuma angustifolia                | 285.3 ± 0.6             | ND  | ND  | ND  | ND  |
| Curcuma Wan Khabitong               | 6.8 ± 0.1               | ND  | ND  | 4.4 ± 0.2 | ND  |
| Curcuma Wan Patab                   | 31.2 ± 0.3              | 2.5 ± 0.0 | ND  | 0.2 ± 0.0 | ND  |
| Curcuma Wan Kortong                 | ND                      | ND  | ND  | 3.5 ± 0.2 | ND  |
| Curcuma Wan Na-Natong               | 144.3 ± 2.8             | 47.1 ± 0.4 | 13.2 ± 0.1 | ND  | ND  |
| Curcuma petiolata                   | ND                      | ND  | ND  | 0.4 ± 0.0 | ND  |
| Curcuma Wan Khamin-Khao-Padtalod    | ND                      | ND  | ND  | 17.6 ± 0.1 | ND  |
| Curcuma Wan Chai-Dam                | ND                      | 65.1 ± 0.2 | 13.5 ± 0.3 | ND  | ND  |
| Curcuma Wan Khamintong              | ND                      | ND  | ND  | 4.6 ± 0.0 | ND  |

1 Based on the weight of ethanol extraction residue from dried Curcuma species (wt%). 2 ND = not detected.

4. Conclusions

The phytochemical property of 23 Curcuma species was studied by means of total phenolic contents and antioxidant activity assays, as well as compositional analysis. Besides, eight bioactive curcuminoids and sesquiterpenoids were isolated from the rhizomes of C. longa, C. comosa, C. latifolia, and C. zedoaria. In particular, the isolation of germacrone (5), furanodienone (6), and zederone (7) is the first report from C. latifolia. From the compositional analysis, C. longa was distinct in its highest curcumin (1) concentration. C. angustifolia was found to contain the highest content of germacrone (5). Out of 23 Curcuma species, five Curcuma species did not contain any curcuminoids 1–4. None of the analyzed Curcuma species contained all eight bioactive compounds. Therefore, the data of phytochemical profiling can be used for the identification and authentication of cultivated Curcuma species. In addition, there is a tremendous potential for the utilization of less-studied Curcuma species as functional foods and ingredients.
Supplementary Materials: The following are available online at http://www.mdpi.com/2304-8158/9/9/1219/s1, Table S1: List of 23 Curcuma species, Figure S1: Spectral data for the isolated compounds, Table S2: Taxonomy of 23 Curcuma species.

Author Contributions: Conceptualization, J.H. and Y.P.; methodology, S.B. and M.K.; validation, M.K., B.E.E., and J.H.; investigation, S.B. and M.K.; resources, S.B. and Y.P.; data curation, S.B.; writing—original draft preparation, J.H.; writing—review and editing, B.E.E. and J.H.; visualization, X.X.; supervision, J.H.; funding acquisition, J.H. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors thank the staff at the Bangkok Herbarium, Thailand for the technical support on the taxonomic study of Curcuma species.

Conflicts of Interest: The authors declare no conflict of interest.

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