Antioxidant Activity of Tropical Ginger Extracts and Analysis of the Contained Curcuminoids

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Antioxidant activities of the rhizomes of nine tropical gingers (*Curcuma aeruginosa*, *Curcuma domestica*, *Curcuma heyneana*, *Curcuma mangga*, *Curcuma xanthorrhiza*, *Zingiber cassumunar*, *Phaeomeria speciosa*, *Alpinia galanga*, and *Amomum kepulaga*) have been measured by thiocyanate and TBA methods in a water/alcohol system after extraction and fractionation with organic solvents. The quantity of three known curcuminoids, one of the potent antioxidant family of ginger species, in the extracts has been analyzed by HPLC. The antioxidant activity of the extracts of the gingers was greater than that estimated from the actual quantity of three known curcuminoids in the extracts.

INTRODUCTION

Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defense of living cells against oxidative damage. Although food industries have used effective synthetic antioxidants, recently consumers of food prefer natural antioxidants to synthetic antioxidants on the basis of the assumption that natural compounds are safe. Many investigators have found different types of antioxidants in various kinds of plants (Larson, 1988). We have been interested in the ginger species as a new source of natural antioxidant, because most ginger rhizomes are used for spices in tropical areas and spices are natural food additives established by the food culture. The rhizome of a popular ginger species, *Zingiber officinale*, is well-known to have potent antioxidant activity, and its antioxidant was isolated by Lee and Ahn (1985). In tropical countries, many kinds of gingers are cultivated and used not only for traditional medicines but also for spices. However, little is known about the antioxidant activity and antioxidant compounds in tropical gingers. Interest in such gingers as an antioxidant source prompted us to investigate them chemically. This paper deals with the antioxidant activity of ginger extracts and the relation between these antioxidant activities and the quantity of known antioxidants in various gingers, curcuminoids (1–3), in the extracts (Figure 1).

MATERIALS AND METHODS

Chemical Material. Three curcuminoids, curcumin (diferuloylmethane, 1), demethoxycurcumin [(*p*-hydroxycinnamoyl)-feruloylmethane, 2], and bisdemethoxycurcumin (*p,p'*-dihydroxydicinnamoylmethane, 3), were obtained, after purification by silica gel TLC (5% MeOH in CH_2Cl_2, Merck Art. 5744), from crude curcumin purchased from Funakoshi Pharmaceutical Ltd., Tokyo. The structures and purities were confirmed by 400-MHz ^1H NMR. α-Tocopherol and 2,6-di-*tert*-butylhydroxytoluene (BHT) were purchased from Wako Pure Chemicals, Osaka. Linoleic acid was purchased from Wako and distilled under N_2 before use.

Plant Material. All rhizomes of tropical gingers (*Curcuma aeruginosa* ROXB., *Curcuma domestica* VAL., *Curcuma heyneana* VAL., *Curcuma mangga* VAL., *Curcuma xanthorrhiza* ROXB., *Zingiber cassumunar* ROXB., *Phaeomeria speciosa* KOORD., *Alpinia galanga* SW., and *Amomum kepulaga* SP.) were cultivated in Tabanan Village, Bali, Indonesia, and were collected in April 1990. They were identified by I. G. P. Tengah.

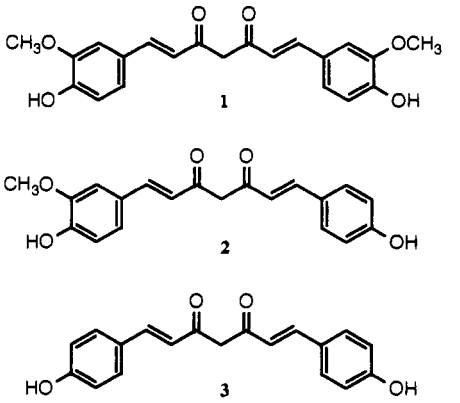

Figure 1. Structures of curcuminoids 1–3.

Extraction and Fractionation. Each fresh rhizome was crushed and soaked for 18 days in acetone (2 L/kg) at 23 °C. After filtration, the solvent was removed under reduced pressure. The residue (the yield is summarized in Table I) was used for antioxidant assays. The residues from *C. domestica*, *A. galanga*, and *C. xanthorrhiza* were used for HPLC analysis. The acetone extracts of *C. aeruginosa*, *C. mangga*, *Z. cassumunar*, and *A. kepulaga* were suspended in water and extracted three times each with *n*-hexane, CH_2Cl_2, and ethyl acetate, successively. All extracts were concentrated under reduced pressure to obtain the materials (the yields are summarized in Table I) for HPLC analysis and antioxidant assays.

Antioxidant Assays. (*1*) *Thiocyanate Method.* The method of Osawa et al. (1981) was slightly modified. A mixture of a sample (4, 2, and 1 mg) in 4 mL of 99.5% ethanol, 4.104 mL of 2.53% linoleic acid in 99.5% ethanol, 8 mL of 0.05 M phosphate buffer (pH 7.0), and 3.896 mL of distilled water was placed in a columnar vial (diameter, 38 mm; height, 75 mm) with a screw cap and placed in an oven at 40 °C in the dark. To 0.1 mL of this sample solution were added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured at 500 nm. Antioxidant activity was judged from inhibition of the absorbance at 1 day before the absorbance of the control vial reached a maximum.

(*2*) *Thiobarbituric Acid (TBA) Method.* The sample solution was prepared and incubated as described above. The TBA value was measured according to Ottolenghi's method (Ottolenghi,

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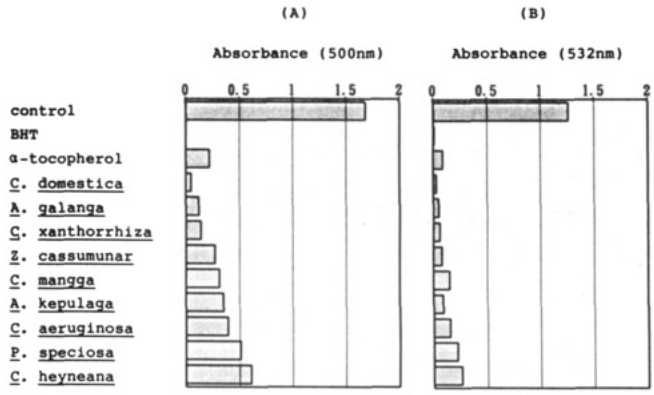

Figure 2. Antioxidant activity of acetone extracts (4 mg) of tropical gingers: (A) thiocyanate method; (B) TBA method. As reference samples, BHT (4 mg) and α-tocopherol (4 mg) were used.

Table I. Yields of Acetone Extracts from Fresh Ginger Rhizomes and Fractionated Yields from Acetone Extracts

	acetone extract, g/100 g	mg/g			
		n-hexane	CH_2Cl_2	ethyl acetate	H_2O
C. aeruginosa	2.1	399	84	32	485
C. domestica	3.9				
C. heyneana	4.3				
C. mangga	2.1	74	200	150	576
C. xanthorrhiza	2.2				
Z. cassumunar	4.5	311	142	21	526
P. speciosa	1.0				
A. galanga	2.9				
A. kepulaga	5.6	215	48	64	673

1959). Antioxidant activity was judged from the inhibition of the absorbance at 1 day after the absorbance of the control vial in the above thiocyanate method reached a maximum.

Quantitative Analysis of Curcuminoids 1–3. A Hitachi L6250 pump system attached to a Hitachi L4200 UV–vis detector and a D2500 chromato-integrator was used as a HPLC instrument. A Develosil ODS K-5 column (0.5 mm o.d. × 20 cm, Nomura Chemicals, Kasugai, Japan) attached to a precolumn was used for the analysis. Four milligrams of each extract dissolved in 1 mL of 1% acetic acid in CH_3CN was passed through a Sep-Pak C_{18} cartridge (Waters-Millipore, Milford, MA) with the same solvent to make 5 mL of sample solution, 1 μL of which was used for analysis. The analysis was carried out with a mobile phase of acetic acid/CH_3CN/H_2O (1:55:45 v/v) at a flow rate of 0.5 mL/min. The peaks corresponding to curcuminoids 1–3 were detected at 420 nm after 22, 20, and 18 min, respectively. Each curcuminoid quantity was calculated by comparing the peak area with the following calibration curves obtained by using standard solution of pure curcuminoids (ranging between 0.005 and 0.2 μg): **1**, $y = 5903978x - 16106$ ($r = 0.99$); **2**, $y = 6426245x - 8895$ ($r = 0.99$); **3**, $y = 4751579x + 474$ ($r = 0.99$); y is peak area and x is each curcuminoid quantity (μg/μL).

RESULTS AND DISCUSSION

Antioxidant Activity of the Ginger Extracts. As shown in Figure 2, acetone extracts of all gingers showed antioxidant activity. The antioxidant activity increased in the order *C. heyneana* < *P. speciosa* < *C. aeruginosa* < *A. kepulaga* < *C. mangga* < *Z. cassumunar* < *C. xanthorrhiza* < *A. galanga* < *C. domestica* by the thiocyanate method and in the order *C. heyneana* < *P. speciosa* < *C. mangga* = *C. aeruginosa* < *A. kepulaga* < *Z. cassumunar* < *C. xanthorrhiza* < *A. galanga* < *C. domestica* by the TBA method. Especially, the activities of the extracts of *C. domestica*, *A. galanga*, and *C. xanthorrhiza* were stronger than that of α-tocopherol. The extracts of *C. aeruginosa*, *C. mangga*, *A. kepulaga*, and *Z. cassumunar* showed a moderate antioxidant activity, which was slightly lower than that of α-tocopherol. It should be noted that some of the ginger rhizomes have a large amount of essential oils (Hegnauer, 1963), which promote increased peroxide value to our knowledge (Nakatani et al., 1986). We thought that the activity of antioxidant compounds contained in such gingers having moderately active extracts would be detected clearly after removal of the essential oil. The removal of the essential oil from moderately active extracts was carried out by extraction with *n*-hexane from the suspension of the acetone extracts in water. After extraction with *n*-hexane, the residual solution was successively extracted with CH_2Cl_2 and ethyl acetate to separate the CH_2Cl_2, ethyl acetate, and H_2O soluble fractions. The antioxidant activity of the fractions was measured, and data are shown in Figure 3. Strong antioxidant activity was clearly detected in all moderately polar, CH_2Cl_2, and ethyl acetate soluble fractions, except for *C. mangga*. The antioxidant activity increased in the order *C. aeruginosa* (H_2O extr) < *A. kepulaga* (H_2O extr) < *C. mangga* (CH_2Cl_2 extr) < *Z. cassumunar* (H_2O extr) < *C. mangga* (H_2O extr) < *C. mangga* (ethyl acetate extr) < *Z. cassumunar* (ethyl acetate extr) < *Z. cassumunar* (CH_2Cl_2 extr) = *A. kepulaga* (CH_2Cl_2 extr) = *A. kepulaga* (ethyl acetate extr) < *C. aeruginosa* (CH_2Cl_2 extr) < *C. aeruginosa* (ethyl acetate extr) by the thiocyanate method and in the order *C. mangga* (CH_2Cl_2 extr) < *C. aeruginosa* (H_2O extr) < *A. kepulaga* (H_2O extr) < *Z. cassumunar* (H_2O extr) < *C. mangga* (H_2O extr) < *C. mangga* (ethyl acetate extr) < *C. aeruginosa* (CH_2Cl_2 extr) < *Z. cassumunar* (CH_2Cl_2 extr) = *A. kepulaga* (CH_2Cl_2 extr) < *Z. cassumunar* (ethyl acetate extr) = *C. aeruginosa* (ethyl acetate extr) < *A. kepulaga* (ethyl acetate extr) by the TBA method. Recently, the potent antioxidant activity of three known curcuminoids isolated from *C. longa* was reported by Toda et al. (1985). They reported the activity of curcumin (**1**) and its analogs (**2** and **3**) to be much stronger than that of α-tocopherol by their method. The activity of the extracts indicated to us that the antioxidants in the extracts were curcuminoids, because they were known to exist widely in ginger species (Jentszch et al., 1970) and have the soluble polarity in CH_2Cl_2 and ethyl acetate. To clarify the contribution of the curcuminoids to the antioxidant activity of the extracts, we tried quantitative analysis of the curcuminoids in strong active fractions of the ginger extracts.

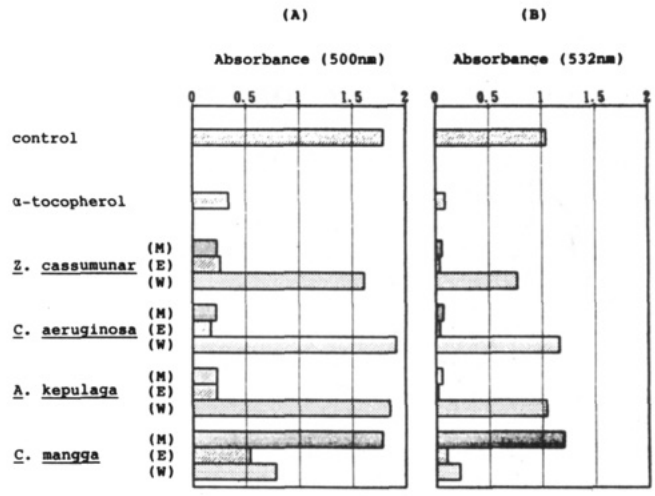

Figure 3. Antioxidant activity of CH_2Cl_2 (M), ethyl acetate (E), and H_2O (W) extracts (4 mg) of the gingers having moderate antioxidant activity: (A) thiocyanate method; (B) TBA method. As a reference sample, α-tocopherol (4 mg) was used.

Analysis of Curcuminoids 1–3 in the Extracts. Curcuminoids have received attention not only as food coloring

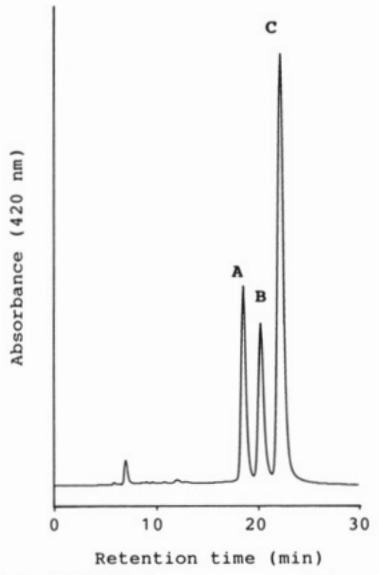

Figure 4. Typical HPLC chromatogram of the acetone extract of *C. domestica*: (A) bisdemethoxycurcumin (3); (B) demethoxycurcumin (2); (C) curcumin (1).

reagents but also because of attractive biological activities such as inhibition of tumor promotion (Huang et al., 1991). Quantitative analysis of the curcuminoids has been difficult, because the curcuminoids are unstable and absorbed irreversibly on various chromatography supports. Recently, several analytical methods for curcuminoids by HPLC have been reported (Asakawa et al., 1981; Tønnensen and Karlsen, 1983, 1986; Rouseff, 1988). We used HPLC for the quantitative analysis of three curcuminoids in our crude extracts. As for the accuracy of the quantitative analysis of curcuminoids, recovery was an important problem. A reversed-phase column was useful, considering the property of the curcuminoids. We chose an ODS column (Nomura Chemical, Develosil ODS-5), by which three curcuminoids could be separated with 55% CH_3CN in H_2O as an eluent. However, the recovery under these conditions was poor (under 70%). Asakawa et al. (1981) reported the analysis of curcumin (1) by HPLC with acetic acid containing solvent as an eluent. Addition of acetic acid (1%) to the eluent sharpened the peaks of the curcuminoids and raised their recovery to 95% (Figure 4). This acetic acid containing eluent was also effective in pretreatment of crude extracts with a Sep-Pak C_{18} cartridge. Under these conditions, a linear slope was obtained as a standard curve between the integrated peak area and each curcuminoid quantity. The curcuminoid quantity in the ginger extracts was measured according to the established HPLC method; data are shown in Table II. The acetone extracts of *C. domestica* and *C. xanthorrhiza* and the CH_2Cl_2 extract of *Z. cassumunar* contain large quantities of curcuminoids. The quantities of curcuminoids in the other extracts were very low or could not be estimated by our method.

Relation between Antioxidant Activity and Curcuminoid Quantity. Toda et al. (1984) reported that the antioxidant activities of curcuminoids 1–3 were 20, 9, and 8 times, respectively, stronger than that of α-tocopherol with respect to peroxide value by their modified active oxygen method. This strong activity of curcuminoids suggests that curcuminoids are the antioxidant principles in the ginger extracts having a high quantity of curcuminoids. We tried to evaluate the activity of the curcuminoids in the alcohol/water system we used; data are shown in Figure 5. Three curcuminoids showed almost the same efficiency, and their activity was ca. 2.5 times

Table II. Curcuminoid Quantity in 4 mg of Ginger Extracts, Used for Antioxidant Assay, Determined by a HPLC Method

	content, μg/4 mg		
	1	2	3
C. domestica			
acetone extr	494	129	187
C. xanthorrhiza			
acetone extr	302	87	37
Z. cassumunar			
CH_2Cl_2 extr	384	tr[a]	–[b]
ethyl acetate extr	38	–	–
C. aeruginosa			
CH_2Cl_2 extr	tr	tr	–
ethyl acetate extr	tr	–	–
A. kepulaga			
CH_2Cl_2 extr	tr	–	–
ethyl acetate extr	tr	–	–
A. galanga			
acetone extr	tr	tr	–

[a] tr, trace amount (<25 μg/4 mg). [b] –, not detected.

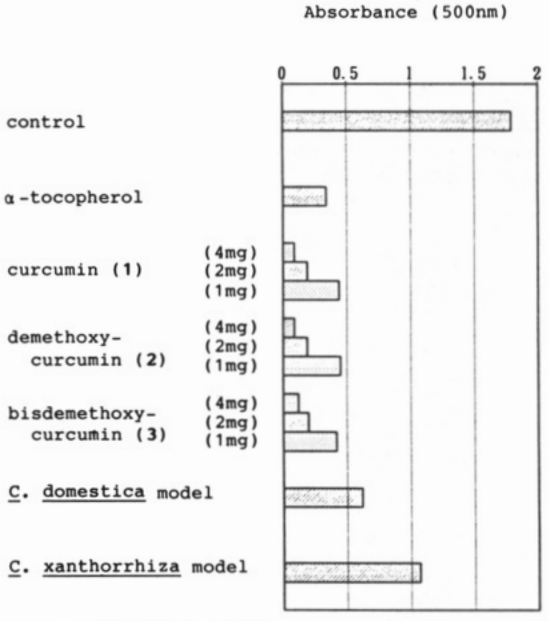

Figure 5. Antioxidant activity of curcuminoids 1–3 and their mixture (*C. domestica* model, 1 490 μg + 2 130 μg + 3 190 μg; *C. xanthorrhiza* model, 1 300 μg + 2 90 μg + 3 40 μg) by a thiocyanate method. As a reference sample, α-tocopherol (4 mg) was used.

stronger than that of the reference sample, α-tocopherol. The synergistic interaction of the three curcuminoids was negligible on the basis of antioxidant activity of the model system for the quantity of the three curcuminoid in *C. domestica* and *C. xanthorrhiza* (1 490 μg + 2 130 μg + 3 190 μg; 1 300 μg + 2 90 μg + 3 40 μg, respectively) (Figure 5). The data indicate that the quantity of the curcuminoids in the extracts estimated from HPLC analysis yielded much lower activity than that of α-tocopherol. The strong antioxidant activity of these ginger extracts cannot be explained only by these curcuminoid contents not only in the low curcuminoid extracts but also in the high curcuminoid extracts. Thus, tropical ginger extracts have additional antioxidants aside from the three known curcuminoids. Our project for finding additional new antioxidants in ginger extracts is now in progress.

Conclusion. The extracts from nine tropical gingers showed antioxidant activity. The acetone extracts from *C. domestica*, *A. galanga*, and *C. xanthorrhiza* and the moderate polar fractions of the extracts from three gingers (*Z. cassumunar*, *C. aeruginosa*, and *A. kepulaga*) have

strong activity, which can replace α-tocopherol as a naturally occurring antioxidant. All examined ginger extracts had a low curcuminoid content in spite of their strong activity, which indicated the possibility of finding a new effective antioxidant in the tropical gingers.

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LITERATURE CITED

Asakawa, N.; Tsuno, M.; Hattori, T.; Ueyama, M.; Shinoda, A.; Miyake, Y.; Kagei, K. Determination of Curcumin Content of Turmeric by High Performance Liquid Chromatography. *Yakugaku Zasshi* **1981**, *101*, 374–377.

Hegnauer, R. Zingiberaceae. In *Chemotaxonomie der Pflanzen*; Birkhäuser Verlag: Basel, 1963; Vol. 2, pp 451–471.

Huang, M. T.; Lysz, T.; Ferraro, T.; Wang, Z. Y.; Conney, A. H. Inhibitory Effects of Curcumin on Tumor Initiation and Promotion in Mouse. *Abstracts of Papers*, Fourth Chemical Congress of North America, New York; American Chemical Society: Washington, DC, 1991; AGFD88.

Jentszch, K.; Spiegel, P.; Kamitz, R. Qualitative and Quantitative Studies of Curcuma Dyes in Different Zingiberaceae Drugs 2. Quantitative Studies. *Sci. Pharm.* **1970**, *38*, 50–58.

Larson, R. A. The Antioxidants of Higher Plants. *Phytochemistry* **1988**, *27*, 969–978.

Lee, I.; Ahn, S. The Antioxidant Activity of Gingerol. *Korean J. Food Sci. Technol.* **1985**, *17*, 55–59.

Nakatani, N.; Inatani, R.; Ohta, H.; Nishioka, A. Chemical Constituents of Peppers (*Piper* spp.) and Application to Food Preservation: Naturally Occurring Antioxidative Compounds. *Environ. Health Perspect.* **1986**, *67*, 135–142.

Osawa, T.; Namiki, M. A Novel Type of Antioxidant Isolated from Leaf Wax of *Eucalyptus* Leaves. *Agric. Biol. Chem.* **1981**, *45*, 735–739.

Ottolenghi, A. Interaction of Ascorbic Acid and Mitochondrial Lipides. *Arch. Biochem. Biophys.* **1959**, *79*, 355–363.

Rouseff, R. L. High Performance Liquid Chromatographic Separation and Spectral Characterization of the Pigments in Turmeric and Annatto. *J. Food Sci.* **1988**, *53*, 1823–1826.

Toda, S.; Miyase, T.; Arichi, H.; Tanizawa, H.; Takino, Y. Natural Antioxidants III, Antioxidative Components Isolated from Rhizome of *Curcuma longa* L. *Chem. Pharm. Bull.* **1985**, *33*, 1725–1728.

Tønnensen, H. H.; Karlsen, J. High-performance Liquid Chromatography of Curcumin and Related Compounds. *J. Chromatogr.* **1983**, *259*, 367–371.

Tønnensen, H. H.; Karlsen, J. Studies on Curcumin and Curcuminoids. *Z. Lebensm. Unters. Forsch.* **1986**, *182*, 215–218.

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