Antioxidative Activity of Carbazoles from *Murraya koenigii* Leaves

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The antioxidative properties of the leaves extracts of *Murraya koenigii* using different solvents were evaluated based on the oil stability index (OSI) together with their radical scavenging ability against 1-1-diphenyl-2-picrylhydrazyl (DPPH). The methylene chloride (CH_2Cl_2) extract and the ethyl acetate (EtOAc) soluble fraction of the 70% acetone extract significantly prolonged the OSI values comparable to those of α-tocopherol and BHT. Five carbazole alkaloids were isolated from the CH_2Cl_2 extract and their structures were identified to be euchrestine B (**1**), bismurrayafoline E (**2**), mahanine (**3**), mahanimbicine (**4**), and mahanimbine (**5**) based on ^1H and ^{13}C NMR and mass (MS) spectral data. The OSI value of carbazoles at 110 °C decreased in the order **1** and **3** > α-tocopherol > BHT > **2** > **4**, **5** and control. It is assumed that compounds **1** and **3** contributed to the high OSI value of the CH_2Cl_2 extract of *M. koenigii*. The DPPH radical scavenging activity for these carbazoles was in the order ascorbic acid > **2** > **1**, **3** and α-tocopherol > BHT > **4** and **5**.

Keywords: *Carbazole; Murraya koenigii; curry leaf; antioxidant; oil stability index (OSI); 1-1-diphenyl-2-picrylhydrazyl (DPPH)*

INTRODUCTION

The plant *Murraya koenigii* (L.) Spreng. belonging to the family Rutaceae is native to India and now distributed in most of southern Asia. The leaves of this plant are well-known as curry leaves and have been used as one of the important herbs of southern Indian cooking. *M. koenigii* has also been used as a folk medicine. The leaves increase digestive secretions and relieve nausea, indigestion, and vomiting (*1*). Phytochemical studies on the leaves, stem bark, and root of this plant have resulted in the isolation of carbazole alkaloids (*2*−*8*).

Another source of carbazoles is microorganisms. Carbazole compounds proved to be a major class of antioxidant produced by *Streptomyces* (*9*). Carazostatin, 1-heptyl-3-hydroxy-2-methylcarbazole, from *Streptomyces chromofuscus* showed antioxidant activity against the oxidation of rat brain homogenate induced by iron (II) and ascorbic acid (*10*). Iwatsuki et al. (*11*) reported that carazostatin acted as a strong antioxidant against oxidation of methyl linoleate and soybean phosphatidylcholine liposomes. Neocarazostatins A, B, and C which were isolated from *Streptmyces* sp. strain GP38 showed strong inhibitory activities against lipid peroxidation induced by free radicals in rat brain homogenates (*12*). Carbazoquinocins A to F from *Streptomyces violaceus* showed strong inhibitory activity against lipid peroxidation induced by free radicals in rat liver microsomes preparations free from vitamin E (*13*).

Only a few studies have involved antioxidative activity of carbazoles from *M. koenigii*. Ramsewak et al. (*14*) reported antioxidant activity and other biological activities of mahanimbine, murrayanol, and mahanine isolated from *M. koenigii*. Murrayanol and mahanine showed very weak antioxidant activity, and mahanimbine showed less active than *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) against model liposome oxidation using fluorescence spectroscopy.

We previously established the model oil system for the determination of antioxidative activity by oil stability index (OSI) method (*15*). In the preliminary investigation, we found that extracts of *M. koenigii* showed very strong antioxidative activity in comparison with BHT and α-tocopherol evaluated based on OSI values. The purpose of this study is to examine antioxidants from the leaves of *M. koenigii* and to evaluate antioxidant activity of the extracts and compounds isolated from *M. koenigii* by measuring OSI value and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

MATERIALS AND METHODS

Materials. Methyl linoleate (95% grade) was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). α-Tocopherol, BHT, DPPH, acetone, benzene, chloroform, EtOAc, hexane, CH_2Cl_2, and methanol (MeOH) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Silicone oil (TSF451-100) was purchased from Toshiba Silicone Co. Ltd. (Tokyo, Japan).

General Experimental Procedures. The ^1H, ^{13}C, and 2D NMR spectra were taken by a Varian Unity 500 (500 MHz) spectrometer using TMS as internal standard (Varian Inc., Palo Alto, CA). EI and HR EIMS were measured at 70 eV on a HITACHI M 2000 mass spectrometer (HITACHI Ltd., Tokyo, Japan). Specific rotations were measured on a JASCO (Tokyo,

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Japan) polarimeter P1030. UV spectra were recorded on a UV-2500PC UV−Vis spectrophotometer (Shimadzu, Kyoto, Japan). Silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex octadecyl silica gel (ODS) DM1020T (Fuji Sylisia Chemical Ltd., Kasugai, Japan) were used for column chromatography, and silica gel 60 F_{254} plates (Merck) were used for TLC. Oxidative stability instrument (Omnion Inc., Rockland, MA) attached to an IBM-compatible computer was used for the OSI measurement. Wallac 1420 multilabel counter (Amersham Pharmacia Biotech AB) as microplate reader was employed for measuring DPPH radical scavenging activity.

Plant Material. Leaves of *Murraya koenigii* were collected in Selangor, Malaysia, in January 1998. A voucher specimen (No. 06367) was deposited at the herbarium of Universiti Putra Malaysia, Selangor, Malaysia.

Extraction and Isolation. Air-dried and ground leaves (400 g) of *M. koenigii* were successively extracted with CH_2Cl_2 (3×2 L) and 70% aqueous acetone (5×2 L) at room temperature. For each extraction, the plant material was soaked in the solvent and allowed to stand overnight. The combined CH_2Cl_2 solutions were evaporated in vacuo to give the CH_2Cl_2 extract (31.8 g). The 70% aqueous acetone layer was concentrated in vacuo, and the resulting aqueous solution was partitioned with EtOAc to give the EtOAc-soluble fraction (9.3 g) and water-soluble fraction (53.6 g). The CH_2Cl_2 extract (12.0 g) was chromatographed over silica gel (200 g) and eluted with a mixture solution of CH_2Cl_2 and acetone of increasing polarity to give 10 fractions. Fraction 5 (0.6 g) eluted with CH_2Cl_2 was rechromatographed on a silica gel column using benzene−acetone (9:1) as an eluent, and following recrystallization with *n*-hexane gave compound **1** (121.7 mg) and compound **2** (9.3 mg). Fraction 7 (0.5 g) eluted with CH_2Cl_2 and was rechromatographed on a silica gel column using benzene−acetone (98:2), followed by repeated column chromatography on Sephadex LH-20 (acetone) to give compound **3** (16.4 mg). Fraction 6 (0.5 g) was rechromatographed on an ODS gel (MeOH/H_2O 98:2) column to give 10 fractions. Fraction 6-10 (101.1 mg) was identified as compound **3** by ^{1}H and ^{13}C NMR and MS spectra. Fraction 3 (0.6 g) of the CH_2Cl_2 extract was rechromatographed on a silica gel column using hexane−CH_2Cl_2 (6:4) to give 11 fractions, followed by column chromatography over silica gel (hexane/acetone 9:1) to give three fractions. Fraction 3-3-3 was subject to ODS gel column chromatography (MeOH/H_2O 9:1) to give compounds **4** (2.9 mg) and **5** (10.1 mg). Their structures were identified to be euchrestine B (**1**), bismurrayafoline E (**2**), mahanine (**3**), mahanimbicine (**4**), and mahanimbine (**5**) based on their ^{1}H and ^{13}C NMR and mass spectral data. The structural formulas of compounds **1**−**5** are shown in Figure 1.

Compound 1. UV λ_{max} (MeOH) nm (log ϵ): 211.8 (4.55), 237.8 (4.68), 264.6 (4.41), 310.4 (4.17), 317.2 (4.16), 330.8 (3.88). ^{1}H NMR: δ 1.57 (3H, br s, 7′-CH_3), 1.62 (3H, br s, 7′-CH_3), 1.87 (3H, d, J = 1.0 Hz, 3′-CH_3), 2.03−2.12 (4H, m, H-4′, H-5′), 2.38 (3H, s, 3-CH_3), 3.62 (2H, d, J = 6.8 Hz, H-1′), 3.90 (3H, s, OCH_3), 4.78 (1H, br s, OH), 5.07 (1H, m, H-6′), 5.32 (1H, qt, J = 1.0, 6.8 Hz, H-2′), 6.80 (1H, s, H-1), 6.83 (1H, d, J = 8.5 Hz, H-6), 7.67 (1H, s, H-4), 7.71 (1H, d, J = 8.5 Hz, H-5), 7.74 (1H, br s, NH), ^{13}C NMR δ 16.2 (3-CH_3), 16.4 (3′-CH_3), 17.7 (7′-CH_3), 23.8 (1′), 25.7 (7′-CH_3), 26.6 (5′), 39.7 (4′), 56.7 (OCH_3), 96.7 (1), 104.6 (6), 111.2 (8), 115.8 (3), 117.1 (5), 117.8 (4a), 117.9 (4b), 121.1 (4),122.4 (2′), 124.0 (6′), 131.7 (7′), 136.5 (3′), 139.6 (9a), 140.3 (8a), 152.1 (2), 154.7 (7), EIMS 70 eV *m*/*z* (rel int.): 363 $[M]^+$ (100), 294 (20), 280 (26), 264 (14), 263 (22), 240 (39), $C_{24}H_{29}NO_2$.

Compound 2. UV λ_{max} (CH_2Cl_2) nm (log ϵ): 238.2 (5.02), 312.0 (4.62), 334.4 (4.41). ^{1}H NMR: δ 1.25 (6H, d, J = 1.2 Hz, 3′-CH_3), 1.42 (6H, br s, 7′-CH_3), 1.56 (6H, br s, 7′-CH_3), 1.43−1.52 (4H, m, H-4′), 1.58−1.64 (4H, m, H-5′), 2.48 (6H, s, 3-CH_3), 3.40 (2H, dd, J = 7.3, 15.5 Hz, H-1′), 3.47 (2H, dd, J = 7.3, 15.5 Hz, H-1′), 3.89 (6H, s, 7-OCH_3), 4.78 (2H, m, H-6′), 5.08 (2H, qt, J = 1.2, 7.3 Hz, H-2′), 5.19 (2H, s, OH), 6.85 (2H, d, J = 8.5, H-6), 7.57 (2H, br s, NH), 7.77 (2H, d, J = 8.5 Hz, H-5) 7.85 (2H, s, H-4), ^{13}C NMR δ 15.2 (3′-CH_3), 16.7 (3-CH_3), 17.5 (7′-CH_3), 23.7 (1′), 25.6 (7′-CH_3), 26.3 (5′), 39.1 (4′), 56.7 (OCH_3), 99.4 (1), 104.9 (6), 111.0 (8), 117.3 (4a), 117.3 (5), 117.4 (3), 118.2 (4b), 121.7 (2′), 122.3 (4), 124.1 (6′), 131.1 (7′), 136.5 (3′), 137.8 (9a), 140.5 (8a), 150.0 (2), 154.8 (7), EIMS 70 eV *m*/*z* (rel int.): 724 $[M]^+$ (100), 479 (3), 362 (3). HR-EIMS 70 eV: found: *m*/*z* 724.4277 $[M]^+$, $C_{48}H_{56}N_2O_4$ requires 724.4237.

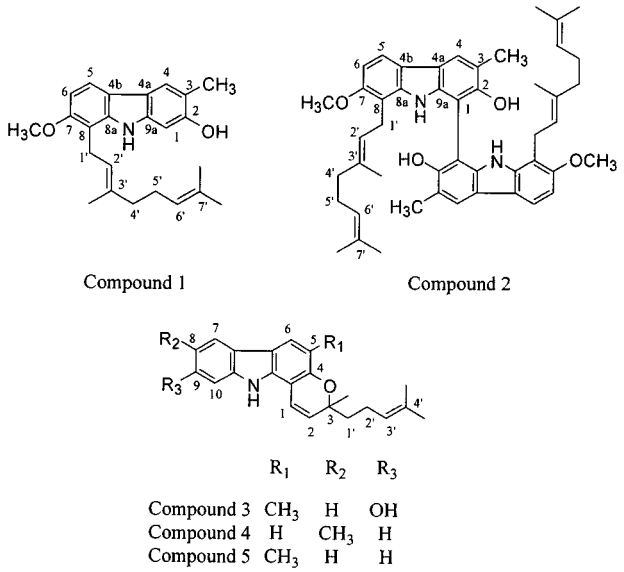

Figure 1. Structural formulas of carbazoles from *Murraya koenigii*.

Compound 3. UV λ_{max} (MeOH) nm (log ϵ): 211.0 (4.60), 240.6 (4.66), 285.4 (4.40, sh), 295.2 (4.56), 325.2 (3.90), 342.2 (3.92), 356.6 (3.86). ^{1}H NMR: δ 1.43 (3H, s, 3-CH_3), 1.57 (3H, br s, 4′-CH_3), 1.65 (3H, br s, 4′-CH_3), 1.72−1.78 (2H, m, H-1′), 2.11−2.20 (2H, m, H-2′), 2.31 (3H, br s, 5-CH_3), 5.01 (1H, br s, OH), 5.11 (1H, m, H-3′), 5.63 (1H, d, J = 9.8 Hz, H-2), 6.57 (1H, d, J = 9.8 Hz, H-1), 6.68 (1H, dd, J = 2.2, 8.3 Hz, H-8), 6.77 (1H, d, J = 2.2 Hz, H-10), 7.54 (1H, s, H-6), 7.71 (1H, d, J = 8.3 Hz, H-7), 7.73 (1H, br s, NH), ^{13}C NMR δ 16.0 (5-CH_3), 17.6 (4′-CH_3), 22.7 (2′), 25.7 (3-CH_3), 25.7 (4′-CH_3), 40.7 (1′), 78.0 (3), 97.0 (10), 104.3 (11b), 108.3 (8), 116.7 (6a), 117.5 (1), 118.0 (7a), 118.3 (5), 120.0 (7), 120.4 (6), 124.2 (3′), 128.7 (2), 131.7 (4′), 134.7 (11a), 140.7 (10a), 148.9 (4a), 153.5 (9), EIMS 70 eV *m*/*z* (rel int.): 347 $[M]^+$ (100), 332 (19), 304 (8), 278 (15), 264 (75), $C_{23}H_{25}NO_2$. $[\alpha]_D^{25}$ = +7.6° ($CHCl_3$; c 5.38).

Compound 4. UV λ_{max} (MeOH) nm (log ϵ): 238.2 (4.61), 280.0 (4.29, sh), 289.6 (4.56), 334.2 (3.78), 353.2 (3.75). ^{1}H NMR: δ 1.45 (3H, s, 3-CH_3), 1.57, 1.65 (each 3H, br s, 4′-CH_3), 1.69−1.82 (2H, m, H-1′), 2.13−2.19 (2H, m, H-2′), 2.50 (3H, s, 8-CH_3), 5.10 (1H, m, H-3′), 5.66 (1H, d, J = 9.8 Hz, H-2), 6.65 (1H, d, J = 9.8 Hz, H-1), 6.71 (1H, d, J = 8.5 Hz, H-5), 7.14 (1H, dd, J = 1.7, 8.3 Hz, H-9), 7.28 (1H, br d, J = 8.3 Hz, H-10), 7.72 (1H, d, J = 1.7 Hz, H-7), 7.74 (1H, d, J = 8.5 Hz, H-6), 7.85 (1H, s, NH), ^{13}C NMR: δ 17.6, (4′- CH_3), 21.5 (8-CH_3), 22.7 (C-2′), 25.7 (4′- CH_3), 26.0 (3-CH_3), 40.8 (C-1′), 78.3 (C-3), 104.5 (C-11b), 109.4 (C-5), 110.1 (C-10), 117.3 (C-1), 117.4 (C-6a), 119.5 (C-7), 120.4 (C-6), 124.1 (C-3′), 124.1 (C-7a), 125.7 (C-9), 128.7 (C-2), 129.0 (C-8), 131.7 (C-4′), 136.6 (C-11a), 137.7 (C-10a), 151.7 (C-4a). EIMS 70 eV *m*/*z* (rel int.): 331 $[M]^{+23}$, 316 (6), 248 (100), 210 (3), $C_{23}H_{25}NO$. $[\alpha]_D^{25}$ = +58.1° ($CHCl_3$; c 0.26).

Compound 5. UV λ_{max} (MeOH) nm (log ϵ): 223.0 (4.56 sh), 238.0 (4.67), 287.6 (4.61), 329.4 (3.88), 342.6 (3.88). ^{1}H NMR: δ 1.45 (3H, s, 3-CH_3), 1.58, 1.66 (each 3H, br s, 4′-CH_3), 1.73−1.79 (2H, m, H-1′), 2.12−2.22 (2H, m, H-2′), 2.33 (3H, s, 5-CH_3), 5.11 (1H, m, H-3′), 5.66 (1H, d, J = 9.8 Hz, H-2), 6.65 (1H, d, J = 9.8 Hz, H-1), 7.17 (1H, br t, J = 8.1 Hz, H-8), 7.30 (1H, br t, J = 8.1 Hz, H-9), 7.37 (1H, br d, J = 8.1 Hz, H-10), 7.66 (1H, s, H-6), 7.87 (1H, br s, NH), 7.91 (1H, br d, J = 7.8 Hz, H-7), ^{13}C NMR: δ 16.1 (5-CH_3), 17.6, (4′- CH_3), 22.7 (C-2′), 25.7 (4′-CH_3), 25.8 (3-CH_3), 40.8 (C-1′), 78.1 (C-3), 104.2 (C-11b), 110.3 (C-10), 116.6 (C-6a), 117.5 (C-1), 118.4 (C-5), 119.3 (C-7), 119.5 (C-8), 121.2 (C-6), 123.9 (C-7a), 124.2 (C-3′), 124.2

(C-9), 128.5 (C-2), 131.7 (C-4′), 134.8 (C-11a), 139.4 (C-10a), 149.9 (C-4a). EIMS 70 eV *m/z* (rel int.): 331 $[M]^+$ (24), 248 (100), 210 (5), $C_{23}H_{25}NO$. $[\alpha]_D^{25} = +30.0°$ ($CHCl_3$; c 0.69).

Evaluation of OSI Value. The oil stability index (OSI) is defined as the point of maximum change of the rate of oxidation of fats and oils under high temperature (*15*, *16*). A stream of air was bubbled into 5 g of oil contained in a reaction tube placed in an electric heating chamber. The effluent air that contained volatile organic acids generated from the oil was collected in another tube containing distilled water (50 mL). The conductivity of the water as oxidation proceeded was measured automatically. Air flow rate was set at 2.5 mL/s for all determinations.

Ten percent methyl linoleate in silicone oil was used as a model substrate oil for measuring OSI values with and without samples. A total of 100 μL of methanol solutions of samples were added to the 5 g of model substrate oil. Concentrations of methanol solution and temperature were as follows: for the CH_2Cl_2 extract, EtOAc-soluble fraction and water-soluble fraction, one mg of tested samples in 100 μL of methanol solution was added to model oil (final concentration: 0.02% of model substrate oil). Their OSI values were measured at 90, 110, and 120 °C. For the 10 fractions derived from the CH_2Cl_2 extract, one mg in 100 μL of methanol solution was added to model oil. The OSI values were measured at 110 °C. For the five compounds isolated from the CH_2Cl_2 extract, the concentration of methanol solution was 1 μmol of tested samples in 100 μL of methanol (final concentration: 0.2 μmol/g of model substrate oil). Their OSI values were measured at 110 °C.

The mixtures were shaken on a Vortex mixer for 30 s under nitrogen. These were then preheated for 30 min without linking the conductivity measurement tubes. Then the reaction tubes were connected to the conductivity measurement tubes and the OSI values were measured. Three replicates of OSI values were carried out on all samples (*15*).

Evaluation of DPPH Radical Scavenging Activity. The scavenging activity of compounds from the CH_2Cl_2 extract on DPPH radicals was measured according to Brand-Williams et al. (*17*) with some modifications (*18*). To a well in a 96-well flat-bottom microplate were added 75 μL of ethanol solution of sample and 75 μL of 200 μM ethanol solution of DPPH (final concentration 100 μM). Samples were prepared in triplicate for each concentration used (final concentrations were 0.1, 1, 3.2, 10, 31.5, 100 μM). A total of 75 μL of ethanol without samples was added as control. After the reaction mixtures were allowed to stand for 30 min at room temperature, the absorbances of each sample were measured at 520 nm against a blank of ethanol without DPPH. All analyses were run in three replicates. DPPH radical scavenging activity was calculated according to the equation: DPPH radical scavenging activity (%) = [(absorbance of control − absorbance of sample)/absorbance of control] × 100. IC_{50} was defined as concentration of compounds, which showed 50% DPPH scavenging activity.

Statistical Analysis. A factorial analysis of variance (ANOVA) with multiple comparisons and linear regression were used utilizing the Stat View program package (SAS Institute, Inc., Cary, NC). Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Evaluation of OSI Value of Extracts from *M. koenigii*. Table 1 shows the OSI values of the CH_2Cl_2 extract, the EtOAc-soluble fraction and the water-soluble fraction, as compared with α-tocopherol and BHT. The OSI values were measured at 90, 110, and 120 °C at a sample concentration of 0.02% (*15*). Control data are the OSI values of the model oil without any additives. Larger OSI values suggest stronger antioxidative activity against oxidation of model substrate oil under high temperature. The CH_2Cl_2 extract, the EtOAc-soluble fraction, α-tocopherol, and BHT prolonged the OSI values significantly as compared to the control at each temperature. The activity of the CH_2Cl_2 extract and the EtOAc soluble fraction were comparable to those of α-tocopherol and BHT at the temperatures of 90 and 110 °C. At 120 °C, these extracts showed equal values to BHT. The water-soluble fraction had no antioxidative activity under these conditions. The OSI values tested at 90, 110, and 120 °C showed the ranges of 6−60 h, 1−10 h and 0.5−5 h, respectively. As it is recommended that OSI values should be in the range of 4 to 20 h (*15*, *16*), they were measured at 110 °C in the following experiment.

Table 1. OSI Values[a] of Extracts[b] from *Murraya koenigii*, α-Tocopherol, and BHT

samples	OSI (h) 90 °C	110 °C	120 °C
CH_2Cl_2 extract	50.03± 8.97[a]	9.48 ± 0.14[a]	4.75 ± 0.25[a]
EtOAc-soluble fraction	60.68 ± 12.59[a]	8.28 ± 0.90[a]	4.30 ± 0.33[a]
H_2O-soluble fraction	7.75± 1.84[b]	1.65 ± 0.15[b]	0.53 ± 0.03[b]
α-tocopherol	55.38± 5.88[a]	10.52 ± 1.46[a]	5.38 ± 0.19[c]
BHT	39.88± 1.06[a]	9.33 ± 0.16[a]	4.60 ± 0.09[a]
control	6.03± 1.99[b]	1.95 ± 0.35[b]	0.58 ± 0.06[b]

[a] OSI values are mean ± standard deviation of three replicates at 90, 110, and 120 °C. [b] Values in each column with the different superscripts are significantly ($P < 0.05$) different by analysis of variance with multiple comparisons. [c] Samples are added at 0.02% of the oil weight.

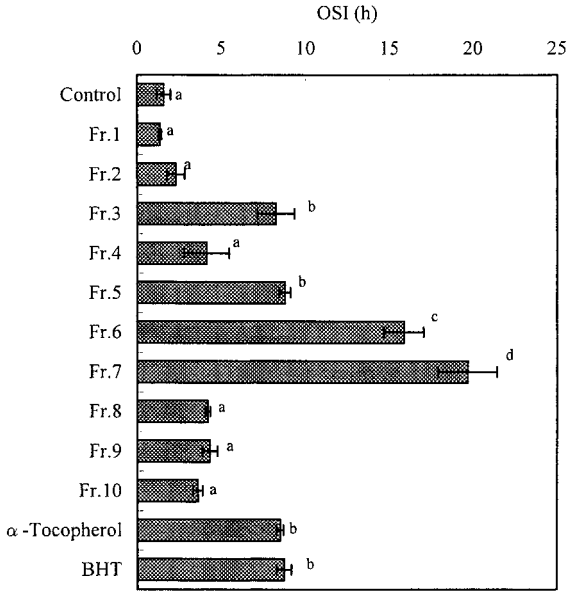

Figure 2. OSI values of the fractions from the CH_2Cl_2 extract of *Murraya koenigii* at 110 °C. (a) OSI values are mean ± standard deviation of three replicates at 110 °C. (b) Values in each column with the different superscripts are significantly ($P < 0.05$) different by analysis of variance with multiple comparisons. (c) Samples are added at 0.02% of the oil weight.

Evaluation of OSI Value of Fractions from the CH_2Cl_2 Extract. The active CH_2Cl_2 extract was chromatographed over silica gel and eluted with CH_2Cl_2 and acetone mixture of increasing polarity to give 10 fractions.

Figure 2 showed the antioxidative activity of the fractions from the CH_2Cl_2 extract measured by OSI method at 110 °C. The activity decreased in the order fraction 7 > fraction 6 > fraction 3, fraction 5, α-tocopherol and BHT > fraction 1, fraction 2, fraction 4, fraction 8, fraction 9, fraction 10, and control.

Isolation and Structure Analysis of Compounds 1−5. The active fraction 5, which showed equal antioxidative activity to α-tocopherol and BHT, was further purified by silica gel column chromatography and recrystallization to give compounds **1** and **2**. The most active fraction (fraction 7) was repeatedly chromatographed on silica gel and Sephadex LH-20 column chromatography to give compound **3**. Fraction 6 was rechromatographed over an ODS gel to yield compound **3**. From fraction 3, compounds **4** and **5** were obtained by silica gel column chromatography.

The UV spectrum of each isolated compound revealed a typical absorption of a carbazole skeleton (*19*, *20*). The molecular formula of compound **1** was calculated as $C_{24}H_{29}NO_2$ based on a combination of EI MS spectrum and ^{13}C NMR spectrum. The ^1H NMR spectrum showed the presence of an NH-proton at δ 7.74, AB-type doublets at δ 6.85 and 7.77 (each $J = 8.5$ Hz) due to ortho-located aromatic protons, and two signals of aromatic protons at δ 6.80 and 7.67. The lower-field signals of δ 7.67 and 7.77 in the ^1H NMR spectrum were deduced for the characteristic signals of H-4 and H-5 in the carbazole nucleus (*19, 20*). The ^1H NMR and double resonance ^1H NMR spectra suggested the presence of a geranyl moiety in the molecule [δ 1.57 (3H, br s), 1.62 (3H, br s), 1.87 (3H, d, $J = 1.0$ Hz), 2.03−2.12 (4H, m), 3.62 (2H, d, $J = 6.8$ Hz), 5.07 (1H, m) and 5.32 (1H, qt, $J = 1.0$, 6.8 Hz)], which was supported by the mass fragment ion peaks at *m/z* 240 [M − (CH═C(CH_3)-CH_2CH_2CH═C(CH_3)$_2$)] in the EI MS spectrum. The ^1H NMR spectrum showed the presence of a phenolic hydroxyl at δ 4.78, a phenolic methoxy at δ 3.90, and a phenolic methyl substituents at δ 2.38. ^1H-detected multiple quantum coherence (HMQC), ^1H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC), and NOESY measurements allowed complete assignments of protons and carbons and determination of the location of substituents on the ring of the carbazoles. These spectral data suggested compound **1** to be euchrestine-B, which was previously isolated from *Murraya euchrestifolia* (*21*). To our knowledge, this is the first isolation of euchrestine-B (**1**) from the leaves of *M. koenigii*.

The molecular formula of compound **2** was established as $C_{48}H_{56}N_2O_4$ by HR MS spectrometry. The ^1H and ^{13}C NMR spectra showed just half signals of its protons and carbons expected from the molecular formula, indicating that this compound had a completely symmetrical structure. The ^1H NMR spectrum was similar to these of **1** except for the lack of H-1 in the ^1H NMR spectrum of **1**. These data suggested the structure of **2** corresponding to a symmetrical dimmer of **1** with the linkage of carbons between C-1 and C-1′. Compound **2** was identified as bismurrayafoline E, which was isolated from the leaves of *M. koenigii* (*22*) by comparison of its ^1H and ^{13}C NMR spectra.

Compound **3** had the molecular formula $C_{23}H_{25}NO_2$ based on its EI MS and ^{13}C NMR spectra. In the aromatic region of the ^1H NMR spectrum, an ABX type system at δ 7.71 (d, $J = 8.3$ Hz), 6.68 (dd, $J = 2.2$, 8.3 Hz), and 6.77 (d, $J = 2.2$ Hz) was observed. The pyranocarbazole skeleton was deduced by an MS spectrum at a high intensity peak of *m/z* 264, which was typical for a carbazolepyrilium ion (*20*). The signals of two *cis*-olefinic protons at δ 5.63 and 6.57 (each d, $J =$ 9.8 Hz) due to a pyran ring annulated to a carbazole moiety belong to H-1 and H-2. The ^1H NMR spectrum showed signals of an NH-proton at δ 7.73, a phenolic hydroxyl at 5.01, a phenolic methyl at 2.31, and 2-methylpent-2-en-5yl substituents at 1.43 (3H, s), 1.57 (3H, br s), 1.65 (3H, br s), 1.72−1.78 (2H, m), 2.11−2.20 (2H, m), and 5.11 (1H, m). HMQC, HMBC, and NOESY measurements allowed complete assignments of protons and carbons and determination of location of substituents on the carbazolepyran skelton. On the basis of comparison of ^1H NMR spectrum, compound **3** was identical with (+)− mahanine isolated from the leaves of *M. euchrestifolia* by Wu. (*23*) We confirmed the assignment of each carbon signal by the HMQC and HMBC measurements (See Material and Methods). While (−)-mahanine was reported with *M. koenigii* (*3*), this is the first report on the isolation of (+)-mahanine from the leaves of *M. koenigii*.

The molecular formula of compounds **4** was established as $C_{23}H_{25}NO$ by EI MS and ^{13}C NMR spectra. The ^1H NMR signals at δ 5.66 (d, $J = 9.8$ Hz), 6.65 (d, $J = 9.8$ Hz), and carbazolepyrilium ion at *m/z* 248 in the MS spectrum showed that this alkaloid has a pyranocarbazole nucleus in the molecule of **4**. The similar ^1H NMR spectrum of **4** to that of **3** indicated the presence of a methyl group on aromatic ring and 2-methylpent-2-en-5yl substituent in **4**. Furthermore, the ^1H NMR spectrum of **4** showed signals due to a NH-proton at δ 7.85, *ortho*-located aromatic protons at δ 6.71 (d, $J = 8.5$ Hz), 7.74 (d, $J = 8.5$ Hz), and 1,2,4-trisubstituted aromatic protons at δ 7.72 (d, $J = 1.7$ Hz), 7.14 (dd, $J = 1.7$, 8.3 Hz), and 7.28 (br d, $J = 8.3$ Hz). The assignment of carbon signals and location of substituents on the carbazolepyran skelton of **4** was achieved on the basis of ^{13}C NMR, HMQC, and HMBC measurements. Compound **4** was identical with mahanimbicine isolated from the leaves of *M. koenigii* by Narasimahan et al. (*3*) by comparing their ^1H NMR spectra and specific rotations.

Compound **5** had the same molecular formula as **4** and a pyranocarbazole moiety in the molecule from NMR and EIMS spectra. The ^1H NMR signals at δ 7.17 (1H, br t, $J = 8.1$ Hz), 7.30 (1H, br t, $J = 8.1$ Hz), 7.37 (1H, br d, $J = 8.1$ Hz), and 7.91 (1H, br d, $J = 7.8$ Hz, H-7) of **5** deduced for unsubstituted carbazole ring. The ^1H NMR spectrum showed the presence of a deshielded phenolic proton at δ 7.66, which was correlated with the phenolic methyl protons by a NOESY measurement. The assignment of carbon signals and location of substituents on the carbazolepyran skeleton of **5** was achieved on the basis of ^{13}C NMR, HMQC, and HMBC measurements. Compound **5** was identical with mahanimbine previously isolated from the stembark of *M. koenigii* by Chakraborty et al. (*24*) by comparison of their ^1H NMR spectra. Narasimahan et al. also reported mahanimbine from the leaves of the same plant (*3*).

Evaluation of OSI Value of Compounds 1−5. The OSI values of the five compounds isolated from the CH_2Cl_2 extract are shown in Table 2. The OSI values containing compounds **1** and **3** were higher than α-tocopherol or BHT. The activity at 110 °C decreased in the order **1** and **3** > α-tocopherol > BHT > **2** > **4**, **5** and control. It is assumed that compounds **1** and **3** contributed to the high OSI value of the CH_2Cl_2 extract from *M. koenigii*.

Evaluation of DPPH Radical Scavenging Activity of Compounds 1−5. Figure 3 showed the radical scavenging activity on DPPH of compounds **1**−**5**. The scavenging activity rose with increasing amounts of

Table 2. OSI Values[a] and DPPH Radical Scavenging Activities[b] of the Extracts from *Murraya koenigii*, α-Tocopherol, BHT, and Ascorbic Acid

samples	OSI (h)	IC_{50} of DPPH radical scavenging activities (μM)
compound **1**	12.28 ± 0.97[a]	21.77 ± 2.39[a]
compound **2**	3.85 ± 0.05[b]	6.80 ± 1.70b
compound **3**	13.28 ± 0.81[a]	21.97 ± 4.18[a]
compound **4**	1.42 ± 0.02[c]	100<
compound **5**	1.90 ± 0.13[c]	100<
α-tocopherol	6.97 ± 1.62[d]	27.77 ± 3.61[a]
BHT	5.27 ± 0.55[e]	83.17 ± 8.42[c]
ascorbic acid		4.40 ± 1.59[b]
control	1.57 ± 0.21[c]	

[a] OSI values were mean ± standard deviation of three replicates at 110 °C. [b] Values in each column with the different superscripts are significantly ($P < 0.05$) different by analysis of variance with multiple comparisons. Samples are added at 0.2 μmol/g of the oil weight. [c] IC_{50} were mean ± standard deviation of three replicates. Values in each column with different superscripts are significantly ($P < 0.05$) different by analysis of variance with multiple comparisons.

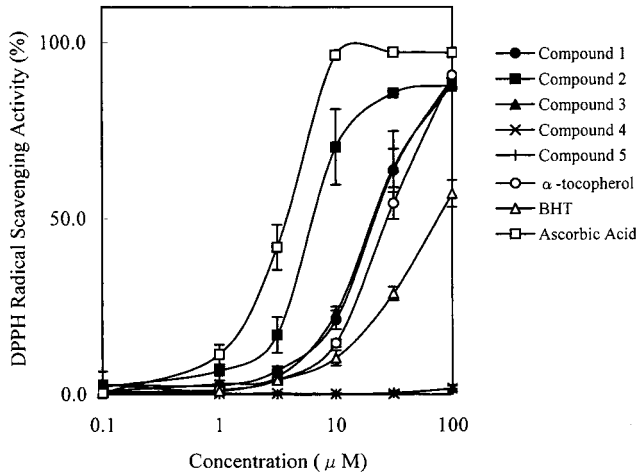

Figure 3. DPPH radical scavenging activity of compounds from *Murraya koenigii* (100 μM DPPH).

compounds, α-tocopherol, and BHT. Table 2 summarized IC_{50} scavenging activity as compared to that of control. The scavenging activity for these compounds was in the order ascorbic acid > **2** > **1**, **3** and α-tocopherol > BHT > **4** and **5**. The DPPH radical scavenging activity of carbazoles increased with the increase in the number of hydroxyl groups. Iwatsuki et al. (*11*) measured antioxidant activities of various natural and synthetic carbazoles in the oxidations of methyl linoleate in homogeneous solution and soybean phosphatidylcholine (PC) liposomes in aqueous dispersion induced by free radicals. They reported that the antioxidant activity required the presence of a free hydroxyl group on the carbazole skeleton such as carazostatin, carbazomycin B, and their derivatives.

Iwatsuki et al. also compared the antioxidant activity of 48 kinds of aminophenols, chromanols, indoles, carbazoles, aromatic amines, and related compounds in the oxidations of methyl linoleate in acetonitrile solution induced by azo radical initiator. They suggested that carbazole did not act as antioxidant per se, but those having either amino or hydroxyl group as a substituent at the *para* position to nitrogen atom acted as potent antioxidant (*25*). On the contrary, 2-hydroxycarbazole, which has a hydroxyl substituent at the *meta* position to nitrogen atom showed no antioxidant activity. However, in this study, compounds **1** and **3**, which have a hydroxyl group at the *meta* position, showed an antioxidant activity against the oxidation of methyl linoleate in bulk system and a DPPH radical scavenging activity.

There was no difference between compounds **1** and **3** (pyranocarbazole skeleton) on the OSI value and DPPH radical scavenging activity. This fact suggests that neither carbazole skeleton nor pyranocarbazole skeleton effects on the degree of activity.

Compound **2**, a dimer of compound **1**, showed the stronger DPPH radical scavenging activity than that of compound **1**. Chen et al. (*26*) reported that rosmarinic acid, a dimer of caffeic acid, also showed the stronger DPPH radical scavenging activity than the monomer. In this study, we found that carbazole possessing two hydroxyl groups such as compound **2** showed more ability to scavenge DPPH radical than carbazole with one hydroxyl group such as compounds **1** and **3**.

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