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Compounds from the Seeds of *Myristica fragrans* and Their Cytotoxic Activity

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Abstract – Six lignan compounds, 1-(17,21-dihydroxyphenyl)-9-(12,13-dihydroxyphenyl)-1-nonanone (malabaricone C) (**1**), 7'-(3',4'-methylenedioxyphenyl)-8,8'-dimethyl-7-(3,4-dihydroxyphenyl)-butane (**2**), 7'-(3',4'-dimethoxyphenyl)-8,8'-dimethyl-7-(3-methoxy-4-hydroxyphenyl)-butane (**3**), 7-(4-hydroxy-3-methoxyphenyl)-7′-(3′,4′-methylenedioxyphenyl)-8,8′-lignan-7-methyl ether (**4**), (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**5**), and (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-acetoxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**6**), were isolated from the seeds of *Myristica fragrans*. The chemical structures of these compounds were determined on the basis of spectroscopic analyses including 2D NMR. Compounds **1 - 6** were evaluated for their cytotoxic activity against the HL-60, MCF-7, and A549 cancer cell lines in *in vitro*.
Keywords – *Myristica fragrans*, Myristicaceae, Lignan, Cytotoxic activity

Introduction

Myristica fragrans Houtt (Myristicaceae) is an aromatic evergreen tree, which is cultivated in South Africa, India and other tropical countries. It has been reported to contain 25 - 30% fixed oils and 5 - 15% volatile oils, such as camphene, elemicin, eugenol, isoelemicin, isoeugenol, methoxyeugenol, pinene, sabinene and safrol, etc., and chemical substances, such as dihydroguaiaretic acid, myristicin and lignans (Janssen *et al*., 1990). Among these compounds, eugenol is widely used in dentistry as root canal sealers and is very effective in their antibacterial activity against oral bacteria (Lai *et al*., 2001). It is also used as local anaesthetic agent for treatment of postoperative pain after gingivectomy (Skoglund and Jorkjend, 1991). The seeds of *M. fragrans* (nutmeg) were imported into Europe during the 12th century and they have long been used indigenously as a spice in many kinds of Western food (Halliwell and Gutteridge, 2000). Nutmeg is also prescribed for medicinal purposes in Asia to treat many diseases, such as rheumatism, muscle spasm, decreased appetite and diarrhea (Kim *et al*., 2007). Nutmeg has shown antioxidant, anti-inflammatory (Jin *et al*., 2005), protein tyrosine phosphatase 1B inhibitory (Yang *et al*., 2006), hepato-protective (Morita *et al*., 2003), and acetylcholine esterase inhibitory activities (Mukherjee *et al*., 2007). However, no study showed the cytotoxic activity from the seeds of *M. fragrans*. This study is part of an ongoing investigation into cytotoxic active compounds from herbal medicines. Extraction and fractionation of *M. fragrans* resulted in the isolation of six compounds (**1 - 6**). This paper describes the isolation, structural elucidation of isolated compounds and their cytotoxic activity against various cancer cell lines.

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Experimental

General experimental procedures – Optical rotations were measured with a JASCO DIP 370 digital polarimeter. UV spectra were taken in MeOH using a Thermo spectrometer, and IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 400 MHz spectrometer. Silica gel (Merck, 63 - 200 µm particle size), RP-18 (Merck, 75 µm particle size) were used for column chromatography. TLC was carried out using Merck silica gel 60 F_{254} and RP-18 F_{254} plates. HPLC was carried out using a Waters system with a UV detector and an YMC Pak ODS-A column (20 × 250 mm, 5 µm particle size, YMC Co., Ltd., Japan) and HPLC solvents were from Burdick & Jackson, USA.

Plant material – The seeds of *M. fragrans* were

purchased in Dong Xuan herbarium market, Hanoi, Vietnam, in January 2010 and identified by Dr Pham Thanh Huyen, Department of Medicinal Plants Resources, National Institute of Medicinal Materials. A voucher specimen (PTH-0110) was deposited in the herbarium of the National Institute of Medicinal Materials, Hanoi, Vietnam.

Extraction and isolation – The seeds of *M. fragrans* (2 kg) were extracted three times (3 h × 3 L) with refluxing methanol. After the solvent was removed under reduced pressure, the residue was suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-BuOH, successively. The EtOAc soluble fraction (54 g) was chromatographed on a silica gel column (10 × 60 cm; 63 - 200 μm particle size, Merck) using a stepwise gradient of $CHCl_3$: MeOH (50 : 1 to 0 : 1, each 2 L) to yield nine fractions (Fr.1 - Fr.9) according to their TLC profiles. Fraction 5 (2.4 g) was subjected to reversed phase (ODS-A) column chromatography (6.0 × 60 cm; 150 μm particle size) and eluted with MeOH/H_2O (from 4:1 to 4:0, 3 L for each step) to afford five sub-fractions (Fr.5-1 to Fr.5-5). Further purification of Fr.5-3 (430 mg) by semi-preparative Waters HPLC systems [using an isocratic solvent system of 70% MeOH in 0.1% trifluoroacetic acid (flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size] resulted in the isolation of compounds **5** (12 mg; t_R = 44.8 min) and **6** (14 mg; t_R = 48.2 min), respectively. Fraction 6 (3.5 g) was also subjected to reversed phase (ODS-A) column chromatography (6.0 × 60 cm; 150 μm particle size) and eluted with MeOH/H_2O (from 5 : 2 to 5 : 0, 2 L for each step) to afford seven sub-fractions (Fr.6-1 to Fr.6-7). Further purification of Fr.6-2 (410 mg) by semi-preparative Waters HPLC systems [using an isocratic solvent system of 65% MeOH in 0.1% trifluoroacetic acid (flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size] resulted in the isolation of compounds **1** (5.7 mg, t_R = 37.2 min). Fraction 7 (3.2 g) was also subjected to reversed phase (ODS-A) column chromatography (6.0 × 60 cm; 150 μm particle size) and eluted with MeOH/H_2O (from 5 : 2 to 5 : 0, 2 L for each step) to afford five sub-fractions (Fr.7-1 to Fr.7-5). Further purification of Fr.7-3 (610 mg) by semi-preparative Waters HPLC systems [using an isocratic solvent system of 70% MeOH in 0.1% trifluoroacetic acid (flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size) resulted in the isolation of compounds **2** (14.5 mg, t_R = 39.2 min) and **3** (23.3 mg, t_R = 49.2 min), respectively. Sub-fraction F.7-4 (359 mg) was further purified by semi preparative HPLC [using an isocratic solvent system of 75% MeOH in 0.1% trifluoroacetic acid (flow rate 5 mL/min) over 90 min; UV detection at 210 nm; YMC Pak ODS-A column (20 × 250 mm, 5 μm particle size) resulted in the isolation compound **4** (21.2 mg, t_R = 45.3 min).

1-(17,21-dihydroxyphenyl)-9-(12,13-dihydroxyphenyl)-1-nonanone (malabaricone C) (1) – White amorphous powder; $[\alpha]_D^{25}$ + 20.0 (*c* 0.1 MeOH); IR ν_{max} (KBr): 3448, 2922, 1629, 1421, 1265, 1163, 1034 cm^{-1}; UV λ_{max} (MeOH): 271 nm; HRTOFMS *m/z* 357.1711 $[M-H]^-$ (calcd for $C_{21}H_{25}O_5$, 357.1702); ^{1}H-NMR (400 MHz, CD_3OD,): δ 3.08 (2H, t, *J* = 7.2 Hz, H-2), 1.63 (2H, m, H-3), 1.25–1.36 (8H, m, H-4 H-7), 1.51 (2H, m, H-8), 2.41 (2H, t, *J* = 7.8 Hz, H-9), 6.61 (1H, br s, H-11), 6.66 (1H, d, *J* = 7.6 Hz, H-14), 6.45 (1H, dd, *J* = 1.2, 8.0 Hz, H-15), 6.32 (2H, d, *J* = 8.0 Hz, H-18 and H-20), 7.15 (1H, t, *J* = 7.2 Hz, H-19); ^{13}C-NMR (100 MHz, CD_3OD,): δ 209.7 (C-1), 45.8 (C-2), 25.7 (C-3), 30.6 (C-4), 30.6 (C-5), 30.5 (C-6), 30.3 (C-7), 32.9 (C-8), 36.3 (C-9), 135.9 (C-10), 116.5 (C-11), 145.9 (C-12), 143.9.0 (C-13), 116.2 (C-14), 120.7 (C-15), 111.4 (C-16), 163.3 (C-17 and C-21), 108.4 (C-18 and C-20), 136.8 (C-19).

7'-(3',4'-methylenedioxyphenyl)-8,8'-dimethyl-7-(3,4-dihydroxyphenyl)-butane (2) – Colorless oil; $[\alpha]_D^{25}$ -9.2 (*c* 0.05, MeOH); UV λ_{max} (MeOH): 284 nm; TOF-MS: *m/z* 313.1 $[M-H]^-$ (calcd. for $C_{19}H_{21}O_4$, 313.1); ^{1}H NMR (400 MHz, CD_3OD) δ : 0.81 (6H, d, *J* = 6.8 Hz, H-9, 9′), 1.72 (2H, m, H-8, 8′), 2.29 (1H, dd, *J* = 8.4, 13.6 Hz, H-7′a), 2.35 (1H, dd, *J* = 8.4, 13.6 Hz, H-7a), 2.47 (1H, dd, *J* = 6.4, 13.6 Hz, H-7′b), 2.54 (1H, dd, *J* = 6.4, 13.6 Hz, H-7b), 6.40 (1H, dd, *J* = 1.6, 8.0 Hz, H-6′), 6.53 (1H, dd, *J* = 1.6, 8.4 Hz, H-6), 6.54 (1H, d, *J* = 1.6 Hz, H-2′), 6.56 (1H, br s, H-2), 6.66 (1H, d, *J* = 8.4 Hz, H-5′), 6.68 (1H, d, *J* = 8.8 Hz, H-5), 5.87 (2H, d, *J* = 1.2 Hz, -OCH_2O); ^{13}C NMR (100 MHz, CD_3OD) δ : 14.3 (2 x CH_3, C-9, 9′), 39.4 (2 x CH_2, C-7, 7′), 42.0 (C-8′), 42.3 (C-8), 108.9 (C-2), 110.3 (C-2′), 116.2 (C-5′), 117.2 (C-5), 121.4 (C-6′), 123.0 (C-6), 134.6 (C-1), 136.9 (C-1′), 144.2 (C-4), 146.0 (C-4′), 147.0 (C-3), 149.0 (C-3′), 102.0 (O$\underline{C}H_2$O).

7'-(3',4'-dimethoxyphenyl)-8,8'-dimethyl-7-(3-methoxy-4-hydroxyphenyl)-butane (3) – Colorless oil; $[\alpha]_D^{25}$ + 20.0 (*c* 0.10, MeOH); UV λ_{max} (MeOH): 282 nm; TOF-MS: *m/z* 345.2 $[M + H]^+$ (calcd. for $C_{21}H_{29}O_4$, 345.2); ^{1}H NMR (400 MHz, CD_3OD) δ : 0.86 (3H, d, *J* = 5.2 Hz, H-9), 0.84 (3H, d, *J* = 5.2 Hz, H-9′), 1.76 (2H, m, H-8, 8′), 2.74 (2H, td, *J* = 3.2, 13.2 Hz, H-7), 2.30 (2H, td, *J* = 5.6, 9.6 Hz, H-7′), 6.68 (1H, d, *J* = 8.0 Hz, H-6′), 6.60 (1H, dd, *J* = 2.0, 8.0 Hz, H-6), 6.69 (1H, s, H-2′), 6.71 (1H, s, H-2), 6.68 (1H, d, *J* = 8.0 Hz, H-5′), 6.85 (1H, d, *J* = 8.0 Hz, H-5), 3.80 (6H, s, 3,3′-OCH_3), 3.78 (3H, s, 4′-OCH_3);

^{13}C NMR (100 MHz, CD_3OD) δ : 16.7 (2 x CH_3, C-9, 9′), 39.9 (C-7), 39.6 (C-7′), 40.2 (C-8′), 40.4 (C-8), 113.1 (C-2), 113.8 (C-2′), 116.0 (C-5′), 114.1 (C-5), 122.5 (C-6, 6′), 134.7 (C-1), 136.3 (C-1′), 148.9 (C-4), 145.6 (C-4′), 150.4 (C-3), 148.6 (C-3′), 56.7 (3-OCH_3), 56.6 (3′-OCH_3), 56.4 (4′-OCH_3).

7-(4-hydroxy-3-methoxyphenyl)-7'-(3',4'-methylenedioxyphenyl)-8,8'-lignan-7-methyl ether (4) – Colorless oil; $[\alpha]_D^{25}$ + 24.0 (*c* 0.10, MeOH); UV λ_{max} (MeOH): 284 nm; TOF-MS: *m/z* 357.1 [M - H]$^-$ (calcd. for $C_{21}H_{25}O_5$, 357.1); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.06 (3H, d, *J* = 7.2 Hz, H-9), 0.75 (3H, d, *J* = 7.2 Hz, H-9′), 1.77 (1H, m, H-8), 1.49 (1H, m, H-8′), 2.73 (1H, dd, *J* = 3.6, 13.6 Hz, H-7′a), 2.08 (1H, dd, *J* = 10.8, 13.6 Hz, H-7′b), 4.04 (1H, d, *J* = 8.0 Hz, H-7), 6.44 (1H, d, *J* = 8.0 Hz, H-6′), 6.80 (1H, d, *J* = 8.0 Hz, H-6), 6.41 (1H, brs, H-2′), 6.88 (1H, br s, H-2), 6.64 (1H, d, *J* = 8.0 Hz, H-5′), 6.85 (1H, d, *J* = 8.0 Hz, H-5), 5.86 (2H, s, 3′,4′-OCH_2O-), 3.87 (3H, s, 3-OCH_3), 3.18 (3H, s, 7-OCH_3); ^{13}C NMR (100 MHz, CD_3OD) δ: 11.6 (C-9), 18.3 (C-9′), 88.6 (C-7), 37.7 (C-7′), 38.3 (C-8′), 46.2 (C-8), 108.8 (C-2), 110.2 (C-2′), 111.8 (C-5′), 116.0 (C-5), 122.9 (C-6′), 121.8 (C-6), 134.0 (C-1), 137.0 (C-1′), 149.0 (C-4), 147.0 (C-4′), 149.3 (C-3), 147.4 (C-3′), 102.0 (3′,4′-O<u>C</u>H_2O-), 56.4 (3-OCH_3), 56.9 (7-OCH_3).

(+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,3',5'-tetramethoxy-8-*O*-4'-neolignan (5) – Colorless oil; $[\alpha]_D^{25}$ + 18.3 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3442, 2923, 1626, 1427, 1271, 1166, 1035 cm^{-1}; UV λ_{max} (MeOH): 277 nm; TOF-MS *m/z* 387.1 [M – H]$^-$ (calcd for $C_{22}H_{27}O_6$, 387.1); ^{1}H-NMR (400 MHz, CD_3OD): δ 6.93 (1H, br s, H-2), 6.92 (1H, d, *J* = 8.4 Hz, H-5), 6.84 (1H, br d, *J* = 8.4 Hz, H-6), 4.79 (1H, d, *J* = 3.2 Hz, H-7), 4.31 (1H, dq, *J* = 3.2, 6.4 Hz, H-8), 1.26 (3H, d, *J* = 6.4 Hz, H-9), 6.52 (2H, br s, H-2′ and H-6′), 3.36 (2H, d, *J* = 6.8 Hz, H-7′), 6.00 (1H, m, H-8′), 5.08 (1H, dd, *J* = 1.2, 11.2 Hz, H-9′*Z*), 5.13 (1H, dd, *J* = 1.2, 13.6 Hz, H-9′*E*), 3.82 (6H, s, 3,4-diOMe), 3.80 (6H, s, 3′,5′-diOMe); ^{13}C-NMR (100 MHz, CD_3OD): δ 131.9 (C-1), 111.8 (C-2), 150.4 (C-3), 150.2 (C-4), 112.8 (C-5), 120.6 (C-6), 75.5 (C-7), 83.8 (C-8), 14.9 (C-9), 137.8 (C-1′), 106.8 (C-2′,6′), 154.7 (C-3′,5′), 134.8 (C-4′), 41.5 (C-7′), 138.9 (C-8′), 116.2 (C-9′), (3,4-diOMe), 56.5 (3′,5′-diOMe).

(+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-acetoxy-3,4,3',5'-tetramethoxy-8-*O*-4'-neolignan (6) – Colorless oil; $[\alpha]_D^{25}$ + 27.1 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3446, 2920, 1624, 1431, 1268, 1164, 1032 cm^{-1}; UV λ_{max} (MeOH): 277 nm; TOF-MS *m/z* 371.1 [M – OAc]$^+$ (calcd for $C_{22}H_{27}O_5$, 371.1); ^{1}H-NMR (400 MHz, CD_3OD): δ 6.93 (1H, br s, H-2), 6.92 (1H, d, *J* = 8.4 Hz, H-5), 6.84 (1H, br d, *J* = 8.4 Hz, H-6), 5.86 (1H, d, *J* = 3.2 Hz, H-7), 4.46 (1H, dq, *J* = 3.2, 6.4 Hz, H-8), 1.26 (3H, d, *J* = 6.4 Hz, H-9), 6.52 (2H, br s, H-2′ and H-6′), 3.36 (2H, d, *J* = 6.8 Hz, H-7′), 6.00 (1H, m, H-8′), 5.08 (1H, dd, *J* = 1.2, 11.2 Hz, H-9′*Z*), 5.13 (1H, dd, *J* = 1.2, 13.6 Hz, H-9′*E*), 3.82 (6H, s, 3, 4-diOMe), 3.80 (6H, s, 3′,5′-diOMe), 2.18 (3H, s, 7- OCO<u>Me</u>); ^{13}C-NMR (100 MHz, CD_3OD): δ 131.9 (C-1), 111.8 (C-2), 150.4 (C-3), 150.2 (C-4), 112.8 (C-5), 120.6 (C-6), 78.2 (C-7), 81.6 (C-8), 14.9 (C-9), 137.8 (C-1′), 106.8 (C-2′,6′), 154.7 (C-3′,5′), 134.8 (C-4′), 41.5 (C-7′), 138.9 (C-8′), 116.2 (C-9′), 56.7 (3,4-diOMe), 56.5 (3′,5′-diOMe), 21.3 (7-OCOMe), 172.2 (7-O<u>C</u>OMe).

Cytotoxicity Assay – The cancer cell lines were maintained in RPMI 1640, which included L-glutamine with 10% FBS and 2% penicillin-streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxic activity was measured using a modified MTT assay (Kim Van *et al*., 2009). Viable cells were seeded in the growth medium (100 μL) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 150 μM by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to < 0.1%. After standing for 24 h, 10 μL of the test sample was added to each well. The same volume of DMSO was added to the control wells. On removing medium after 48 h of the test sample treatment, MTT (5 mg/mL, 10 μL) was also added to the each well. After 4 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μL). The OD was measured at 570 nm. The IC_{50} value was defined as the concentration of sample that reduced absorbance by 50% relative to the vehicle-treated control.

Results and Discussion

The MeOH extract of the seeds of *M. fragrans* was partitioned into hexane-, EtOAc-, *n*-BuOH, and water-soluble fractions. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of six compounds (**1 - 6**) (Fig. 1). The structures of the compounds were determined as 1-(17,21-dihydroxy-phenyl)-9-(12,13-dihydroxyphenyl)-1-no-nanone (malabaricone C) (**1**), 7'-(3',4'-methylenedioxyphenyl)-8,8'-dimethyl-7-(3,4-dihydroxyph enyl)-butane (**2**), 7'-(3',4'-dimethoxy-phenyl)-8,8'-dimethyl-7-(3-methoxy-4-hydroxyphenyl)-butane (**3**), 7-(4-hydroxy-3-methoxyphenyl)-7'-(3',4'-methylenedioxyph enyl)-8,8'-lignan-7-methyl ether (**4**),

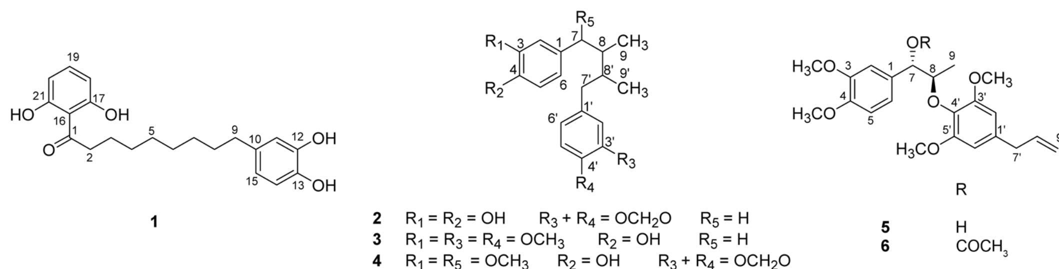

Fig. 1. Chemical structures of isolated compounds **1** - **6**.

(+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-hydroxy-3,4,3',5'-tetramethoxy-8-*O*-4′-neolignan (**5**), and (+)-*erythro*-(7*S*,8*R*)-$\Delta^{8'}$-7-acetoxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**6**) by comparing their physical and spectroscopic data with previous reported papers (Pham *et al*., 2000; Rao and Chattopadhyay, 1990; Miyazawa *et al*., 1997; Kwon *et al*., 2008; Duan *et al*., 2009).

Compounds **1** - **6** were evaluated for their *in vitro* cytotoxic activity against MCF-7, HL-60, and A549 cancer cell lines using MTT assay method with slight modification (Kim Van *et al*., 2009). As the results in table 1, compound **1** (malabaricone C) with the chemical structure as dihydroxyphenyl nonanone skeleton showed the most potent inhibitory activity against A549 cancer cell line with IC_{50} value of 12.3 μM, following by compound 2 with IC_{50} value as 23.4 μM, the other compounds (**3** - **6**) showed weak cytotoxic activity with IC_{50} values over than 30 μM. In the case of HL-60 cancer cell line, compounds **2** and **5** showed the most potent cytotoxic activity with IC_{50} values of 21.0 and 26.1 μM, respectively. Except for the inactivity of **5** and **6**, the remains compounds displayed potential inhibitory activity against MCF-7 cancer cell line IC_{50} values ranging from 10.2 to 15.1 μM.

Malabaricone C (**1**), a natural diarylnonanoids, was isolated the first time from *M. fragrans* exhibited strong antifungal and antibacterial activities (Orabi *et al*., 1991). It irreversibly inhibited Arg-gingipain by 50% at a concentration of 0.7 μM and selectively suppressed *Porphyromomas gingivalis* growth (Shinohara *et al*., 1999). Recently, this compound showed effectively heal indomethacin-induced stomach ulceration in mice by promoting angiogenesis (Banerjee *et al*., 2011), antioxidant (Patro *et al*., 2005), anti-quorum (Chong *et al*., 2011), and anti-inflammation (Maity *et al*., 2012). The anti-cancer activity of this compound and several related chemical structures as malabaricones were also investigated in structure-activity correlation and deduce the mechanistic pathway of the process (Patro *et al*., 2010). Amongst the test compounds, malabaricone C containing a B-ring catechol moiety showed significantly better Cu(II)-dependent nuclease activity than the partially methylated catechol derivatives, it showed better cytotoxic activity than curcumin against the MCF-7 human breast cancer cell line. The malabacinone C-induced killing of the MCF-7 cells followed an apoptotic pathway involving oxidative damage to the cellular DNA (Patro *et al*., 2010). Our results are in accordance with previous publication, and it clearly suggested that the presence of oxygenated substitutions in the aromatic B-ring of the malabaricone C is crucial for their toxicity against the MCF-7 and A549 cells. This is also consistent with a previous finding where the 4"-hydroxyl group in the B-ring has been found to play a key role in the anti-cancer activity of resveratrol (Mello-Filho and Meneghini., 1991; Potter *et al*., 2002). Among 8,8’-hydroxyphenyl lignans, **2** - **4**, the most active compound was **2** (the IC_{50} value of 23.4, 21.0 and 12.3 μM for all the three cell lines, A549, HL-60 and MCF7, respectively). It should be noted that **2** displayed stronger activity than **3** and **4**, this may due to the 3,4-dihydroxyl A-ring is more effective than the 3-methoxy-4-hydroxyl A-ring. It should be mentioned that the cytotoxicity data for these lignans were obtained for the first time in this investigation. In addition, the presence of several lignans in this plant explains its claimed use as a purgative since

Table 1. Cytotoxic activity of isolated compounds against cancer cell lines

Compounds	IC_{50} (μM)		
	A549	HL-60	MCF-7
1	12.3	46.1	10.8
2	23.4	21.0	12.3
3	40.3	> 100	15.1
4	73.5	> 100	10.2
5	> 100	26.1	> 100
6	> 100	67.5	> 100
Adriamycin[a]	2.41	0.87	3.66

[a] Used as positive control.

some lignans are known to have purgative activity, for example, podophyllotoxin from *Podophyllum*, and elenoside from *Justicia hyssopifolia* (Navarro *et al*., 2006). However, the effects of each of these isolates on the intestinal mobility remain to be investigated before any conclusion can be drawn.

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