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Compounds from the Aerial Part of *Saururus chinensis* and Their Cytotoxic Activity

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Abstract – Ten known compounds, 7-hydroxysauchinone (**1**), sauchinone (**2**), di-*O*-methyltetrahydrofuriguaiacin B (**3**), henricine (**4**), saucerneol K (**5**), *meso*-dihydroguaiaretic acid (**6**), (−)-guaiacin (**7**), (3*R*,4*S*)-4-(4-hydroxy-3-methoxyphenyl)-4-methoxy-3-methylbutan-2-one (**8**), (*E*)-7-(4-hydroxy-3-methoxyphenyl)-7-methylbut-8-en-9-one (**9**), and licarin A (**10**), were isolated from aerial part of *Sarurus chinensis*. The chemical structures of these compounds were determined on the basis of spectroscopic analyses including 2D NMR. Compounds **1** - **10** were evaluated for their cytotoxic activity against the HL-60, MCF-7, and A549 cancer cell lines in *in vitro*.
Keywords – *Saururus chinensis*, Saururaceae, Lignan, Cytotoxic activity

Introduction

Saururus chinensis Baill., a perennial herb, belongs to the family Saururaceae, grows throughout East Asia such as China and Korea. It has been used as a medicinal herb to remedy various diseases such as edema, gonorrhea, jaundice, and inflammatory diseases for a long period of time in China and Korea (Chung *et al*., 1990). This plant has been investigated extensively, resulting in the isolation of various lignans (sauchinone, saucerneol, manassantin A, and manassantin B), flavonoids (rutin, hyperoside, quercitrin and quercetin) and alkaloid (Kim *et al*., 2009; Sung, 2006; Kim *et al*., 2004). Previous biological studies of this plant have shown antiasthmatic, antioxidant, and anti-inflammatory (Wang *et al*., 2008), cytotoxic (Park *et al*., 1997), vasorelaxant and inotropic (Ryu *et al*., 2008), hypolipidemic (Yu *et al*., 2008; Yun *et al*., 2007), hypoglycemic (He *et al*., 1992; Joo *et al*., 2006), analgesic (Park *et al*., 1998), neuroprotective (Wie, 2000), antihypertensive (Wang *et al*., 2008), and hepatoprotective effects (Sung and Kim, 2000; Sung *et al*., 1997, Sung *et al*., 2000). *S. chinensis* was also reported to contain compounds such as diterpenes, tannins, steroids, and lipids (Wang *et al*., 2008). Lignans (Sung and Kim, 2000; Sung *et al*., 2000) and flavonol glycosides (Sung *et al*., 1997) from *S. chinensis* were reported to have hepatoprotective effects *in vitro*. Sauchinone, one of the active compounds from this plant, has been reported to inhibit bone destruction and to decrease mortality rate induced by LPS (Han *et al*., 2007; Seo *et al*., 2008). Furthermore, sauchinone attenuated LPS-induced TNF-α, inducible NO synthase and cyclooxygenase-2 gene expression in macrophages stimulated with LPS through the suppression of nuclear translocation of NF-κB (Lee *et al*., 2003; Hwang *et al*., 2003-1). In our continuing investigation to discover cytotoxic compounds, further fractionation of the EtOAc-soluble fraction resulted in the isolation of ten compounds (**1** - **10**). This study describes the isolation, structural elucidation of isolated compounds and evaluation their cytotoxic activity against various cancer cell lines.

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. EI-MS spectrometric data were acquired with a JMS-700 MSTATION mass spectrometer (JEOL, Japan). Silica gel (Merck, 63 - 200

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μm particle size) and 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 performed using a Water 600 Controller system with a UV 486 Tunable Absorbance 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 dried aerial part of *S. chinensis* was purchased from a local folk medicine market named "Yak-ryoung-si" in Daegu, Korea, in May 2010. Botanical identification was performed by Prof. Byung-Sun Min, and the voucher specimen CUD-1384 was deposited at the herbarium of the College of Pharmacy, Catholic University of Daegu, Korea.

Extraction and isolation – The dried aerial part of *S. chinensis* (12 kg) was extracted three times with MeOH at room temperature for seven days and then MeOH extract (1.5 kg) was suspended in hot-water (4 L) and partitioned with *n*-hexane (4 L x 3), ethyl acetate (4 L x 3), and *n*-butanol (4 L x 3), successively. The resulting fractions were concentrated *in vacuo* to give the hexane-soluble fraction (400.8 g), ethyl acetate-soluble fraction (584.93 g), and *n*-BuOH-soluble fraction (314.2 g), respectively. By the activity-guided fractionation, the ethyl acetate-soluble fraction was chromatographed on a silica gel column chromatography eluting with a gradient of $CHCl_3$-MeOH (50 : 1 → 5 : 1) to afford fifteen fractions (Fr. 1~15). Fraction 7 (80 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-acetone (5 : 1 → 0 : 1) to afford three subfractions (Fr. 7-1~7-3). Subfraction 7-1 (20 g) was subjected on a silica gel column chromatography eluting with a gradient of *n*-hexane-EtOAc (10 : 1 → 1 : 1) to afford compounds **2** (120.0 mg), **3** (32.8 mg), and **4** (25.0 mg). Subfraction 7-2 (25 g) was subjected to silica gel column chromatography with a gradient of *n*-hexane-EtOAc (5 : 1 → 1 : 1) to afford seven subfractions (Fr.7-2-1~7-2-7). Subfraction 7-2-5 (450 mg) was chromatographed over a RP-18 gel eluting with a gradient of MeOH-H_2O (3 : 1 → 6 : 1) to afford compounds **6** (34.4 mg), **7** (17.0 mg), **8** (12.3 mg), and **9** (8.0 mg). Subfraction 7-2-2 was chromatographed by MPLC on a ODS column using MeOH-H_2O (3 : 1) and purified by preparative HPLC on a RP-18 column using MeOH- H_2O (72 : 28 → 74 : 26) to yield compound **10** (8.0 mg). Subfraction 7-2-6 was chromatographed by MPLC on a ODS column using MeOH-H_2O (3 : 1) followed by re-crystallization to yield compounds **1** (10.9 mg) and **5** (7.2 mg), respectively.

7-Hydroxysauchinone (1) - White amorphous powder; $[\alpha]_D^{25}$ - 9.5 (*c* 0.37, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 244, 297; IR (KBr) ν_{max} cm^{-1}: 3695, 3019, 1214, 1054, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.60 (3H, d, J = 7.6 Hz, H-9'), 1.25 (3H, d, J = 7.2 Hz, H-9), 1.74 (1H, m, H-7'*ax*), 1.84 (1H, m, H-7'*eq*), 2.06 (1H, m, H-8'), 2.50 (1H, m, H-8), 2.55 (1H, m, H-1'), 2.60 (1H, d, J = 13.2 Hz, H-6'), 5.56 (1H, s, H-3'), 6.43 (1H, s, H-3), 7.0 (1H, s, H-6), 5.97 and 5.94 (each 1H, d, J = 1.2 HZ, 4',5'-OCH_2O-), 5.68 and 5.67 (each 1H, s, 4,5-OCH_2O-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 16.8 (C-9), 20.5 (C-9'), 24.4 (C-7'), 34.9 (C-8'), 40.2 (C-8), 40.5 (C-1'), 44.7 (C-6'), 68.9 (C-7), 99.3 (C-3), 100.7 (C-3'), 100.9 (C-4'), 106.0 (C-6), 119.1 (C-1), 143.8 (C-4), 144.4 (C-2), 148.7 (C-5), 168.2 (C-4'), 198.6 (C-2'). 101.8 (4,5-OCH_2O-), 98.7 (4',5'-OCH_2O-); HR-EI-MS (rel. int.) *m/e*: 372.1209 $[M]^+$ (100), 373.1247 (22) (calcd for $C_{20}H_{20}O_7$; 372.1209).

Sauchinone (2) – White amorphous powder; $[\alpha]_D^{25}$ –85.0 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 244, 300; IR ν_{max} cm^{-1}: 3020, 1214, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.74 (3H, d, J = 7.6 Hz, H-9'), 1.21 (3H, d, J = 7.6 Hz, H-9), 1.67 (1H, m, H-7'*eq*), 1.94 (1H, m, H-7'*ax*), 1.91(1H, m, H-8'), 2.45 (1H, m, H-8), 2.50 (1H, m, H-1'), 2.56 (1H, dd, J = 2.8, 12 Hz, H-6'), 3.05 (1H, d, J = 4.8 Hz, H-7), 5.52 (1H, s, H-3'), 6.40 (1H, s, H-3), 6.85 (1H, s, H-6), 5.62 and 5.68 (each 1H, s, 4',5'-OCH_2O-), 5.89 and 5.93 (each 1H, s, 4,5-OCH_2O-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 20.9 (C-9'), 21.4 (C-9), 25.3 (C-7'), 33.6 (C-8'), 34.9 (C-7), 35.1 (C-8), 35.2 (C-6'), 37.6 (C-1'), 98.7 (4',5'-OCH_2O-), 99.3 (C-3), 100.3 (C-5'), 100.5 (C-3'), 101.4 (4,5 -OCH_2O-), 106.6 (C-6), 115.8 (C-1), 143.3 (C-4), 145.1 (C-2), 146.8 (C-5), 168.7 (C-4'), 199.7 (C-2'); EI-MS (rel. int.) *m/z* : 356 $[M]^+$ (100), 270 (13), 257 (15), 205 (20), 175 (25) (calcd. for $C_{20}H_{20}O_6$).

Di-*O*-methyltetrahydrofuriguaiacin B (3) – White amorphous powder; $[\alpha]_D^{25}$ +28.0 (*c* 0.06, MeOH); UV (EtOH) λ_{max} nm: 241, 281; IR ν_{max} cm^{-1}: 2980, 1214, 748; ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.05 (6H, d, J = 5.6 Hz, H-9,9'), 2.33 (2H, m, H-8,8'), 4.52 (2H, d, J = 5.6 Hz, H-7,7'), 6.87 (2H, d, J = 8.0 Hz H-5,5'), 6.98 (2H, dd, J = 1.6, 8.0 Hz, H-6,6'), 7.0 (2H, d, J = 1.6 Hz H-2,2'), 3.88-3.89 (12H, s, 3, 3', 4, 4'-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 13.2 (C-9,9'), 44.6 (C-8,8'), 87.4 (C-7,7'), 109.9 (C-2,2'), 111.1 (C-5,5'), 118.8 (C-6,6'), 135.0 (C-1,1'), 148.6 (C-4,4'), 149.1 (C-3,3'), 56.1 (3, 3',4,4'-OCH_3); EI-MS (rel. int.) *m/z* : 372 $[M]^+$(25), 206 (100), 191 (75), 175 (70) (calcd. for $C_{22}H_{28}O_5$).

Henricine (4) – Yellow oil; $[\alpha]_D^{25}$ +6.0 (*c* 0.23, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 242, 279; IR (KBr) ν_{max} cm^{-1}: 3696, 3019, 1214, 1054, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.03 (3H, d, J = 6.8 Hz , H-9'), 1.05 (3H, d, J = 7.6 Hz,

H-9), 2.30 (2H, m, H-8,8'), 4.47 (1H, d, J = 6.0 Hz, H-7'), 4.50 (1H, d, J = 6.4 Hz, H-7), 6.63 (1H, s, H-2'), 6.66 (1H, s, H-6'), 6.87 (1H, d, J = 8.0 Hz, H-5), 6.96 (1H, s, H-6), 6.99 (1H, d, J = 4.4 Hz, H-2), 5.96 (2H, s, 3,4-OCH_2O), 3.89 - 3.91 (9H, s, 3',4',5'-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.0 (C-9'), 13.2 (C-9), 44.4 (C-8'), 44.8 (C-8), 87.4 (C-7), 87.6 (C-7'), 100.5 (C-2'), 106.3 (C-6'), 109.9 (C-2), 111.1 (C-5), 118.8 (C-6), 134.7 (C-1), 134.8 (C-4'), 137.3 (C-1'), 143.6 (C-3'), 148.7 (C-4), 149.1 (C-3), 149.2 (C-5') 101.6 (3, 4-OCH_2O-), 56.0 - 56.8 (3',4',5'-OCH_3); EI-MS (rel. int.) *m/z*: 386 $[M]^+$(35), 220 (100), 206 (50), 175 (70) (calcd. for $C_{22}H_{26}O_6$).

Saucerneol K (5) – yellow oil; $[\alpha]_D^{25}$ +15.6 (*c* 0.12, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.06 (3H, d, J = 6.8 Hz , H-9'), 1.08 (3H, d, J = 7.6 Hz, H-9), 2.23 (2H, m, H-8,8'), 4.53 (1H, d, J = 6.0 Hz, H-7'), 4.54 (1H, d, J = 6.4 Hz, H-7), 6.88 (1H, d, J = 1.0 Hz, H-2'), 6.79 (1H, d, J = 8.0, Hz, H-5'), 6.81 (1H, d, J = 8.0, Hz, H-6'), 6.47 (1H, s, H-2), 6.96 (1H, s, H-6), 6.46 (1H, s, H-5), 5.96 (2H, s, 3',4'-OCH_2O), 5.90 (2H, s, 3,4-OCH_2O); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.1 (C-9'), 14.0 (C-9), 48.3 (C-8'), 50.6 (C-8), 89.4 (C-7), 87.5 (C-7'), 106.5 (C-2'), 119.8 (C-6'), 108.2 (C-5'), 99.6 (C-5), 106.8 (C-2), 151.0 (C-6), 134.4 (C-1), 135.4 (C-1'), 147.9 (C-3'), 147.5 (C-4'), 140.7 (C-3), 148.1 (C-4), 101.2 (3', 4'-OCH_2O-), 101.5 (3, 4-OCH_2O-); EI-MS *m/z*: 356 $[M]^+$ (calcd. for $C_{20}H_{20}O_6$).

***meso*-Dihydroguaiaretic acid (6)** – White amorphous powder; $[\alpha]_D^{25}$ -12.8° (*c* 0.07, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 240, 281; IR (KBr) ν_{max} cm^{-1}: 3680, 3019, 1214, 1054, 748, 671; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (6H, d, J = 6.4 Hz, H-9,9'), 1.76 (2H, m, 8,8'-H), 2.39 (2H, dd, J = 7.6, 13.6 Hz, H-7a,7'a), 2.59 (2H, dd, J = 6.8, 13.6 Hz, H-7b,7'b), 5.50 (1H, s, 4,4'-OH), 6.54 (2H, d, J = 1.6Hz, H-2,2'), 6.60 (2H, dd, J = 1.6, 8.0 Hz, H-6,6'), 6.82 (2H, d, J = 8.0 Hz, H-5,5'), 3.85 (6H, s, 3,3'-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.0 (C-9,9'), 37.6 (C-8,8'), 41.3 (C-7,7'), 111.5 (C-2,2'), 114.0 (C-5,5'), 121.8 (C-6,6'), 133.8 (C-1,1'), 143.7 (C-4,4'), 146.4 (C-3,3'), 55.9 (3, 3'-OCH_3); EI-MS (rel. int.) *m/z*: 330 $[M]^+$ (30), 137 (100) (calcd. for $C_{20}H_{26}O_4$).

(–)-Guaiacin (7) – White amorphous powder; $[\alpha]_D^{25}$ –30.0 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} nm: 241, 283; IR (KBr) ν_{max} cm^{-1}: 3694, 3019, 1214, 1032, 748, 671; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.86 (3H, d, J = 6.4 Hz, H-9'), 1.08 (3H, d, J = 6.4 Hz, H-9), 1.53 (1H, m, H-8'), 1.62 (1H, m, H-8), 2.60 (1H, dd, J = 7.2, 16.4 Hz, H-7a), 2.75 (1H, dd, J = 4.4, 16.0 Hz, H-7b), 3.37 (1H, d, J = 10.4 Hz, H-7'), 6.26 (1H, s, H-2), 6.54 (1H, s, H-5), 6.56 (1H, d, J = 2.0 Hz, H-2'), 6.64 (1H, dd, J = 2.0, 8.0 Hz, H-6'), 6.84 (1H, d, J = 8.0 Hz, H-5'), 3.83 (3H, s, 3'-OCH_3), 3.85 (3H, s, 3-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 17.4 (C-9'), 20.2 (C-9), 35.8 (C-8), 39.4 (C-7), 43.9 (C-8'), 54.4 (C-7'), 110.1 (C-5), 111.6 (C-2'), 114.2 (C-5'), 115.7 (C-2), 122.7 (C-6'), 128.5 (C-1), 133.7 (C-6), 138.5 (C-1'), 143.5 (C-4), 144.1 (C-4'), 144.8 (C-3), 146.7 (C-3'), 56.1 (3-OCH_3), 56.2 (3'-OCH_3); EI-MS (rel. int.) *m/z*: 328 $[M]^+$ (100), 241 (85), 271 (40) (calcd. for $C_{20}H_{24}O_4$).

(3*R*,4*S*)-4-(4-Hydroxy-3-methoxyphenyl)-4-methoxy-3-methylbutan-2-one (8) – Yellow amorphous powder; $[\alpha]_D^{25}$ –13.3 (*c* 0.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 239, 281, 332; IR (KBr) ν_{max} cm^{-1}: 3695, 3019, 1214, 1054, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.77 (3H, d, J = 7.2 Hz, 2'-CH_3), 2.26 (3H, s, 3'-CH_3), 2.82 (1H, m, H-2'), 4.12 (1H, d, J = 10.0 Hz, H-1'), 6.77 (1H, dd, J = 1.6, 8.0 Hz, H-6), 6.82 (1H, d, J = 1.6 Hz, H-2), 6.89 (1H, d, J = 8.0 Hz, H-5), 3.10 (3H, s, 1'-OC$\underline{H}_3$), 3.91 (3H, s, 3-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 13.9 (2'-CH_3), 30.1 (3'-CH_3), 53.6 (C-2'), 86.5 (C-1'), 109.1 (C-5), 114.1 (C-2), 121.4 (C-6), 131.3 (C-1), 145.7 (C-4), 147.1 (C-3), 212.3 (C-3'), 56.1 (3-OCH_3), 56.7 (1'-OCH_3); EI-MS (rel. int.) *m/z*: 238 $[M]^+$ (24), 167 (100), 152 (30) (calcd. for $C_{13}H_{18}O_4$).

(*E*)-7-(4-Hydroxy-3-methoxyphenyl)-7-methylbut-8-en-9-one (9) – Yellow amorphous powder; $[\alpha]_D^{25}$ + 8.3 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm: 240, 318; IR (KBr) ν_{max} cm^{-1}: 3696, 3019, 1214, 1054, 749; ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.06 (3H, d, J = 1.2 Hz 2'-CH_3), 2.45 (3H, s, 3'-CH_3), 6.87 (1H, d, J = 8.4 Hz, H-5), 7.04 (1H, dd, J = 2.0, 8.4 Hz, H-6), 7.10 (1H, d, J = 2.0 Hz, H-2), 7.61 (1H, s, H-1'), 3.89 (3H, s, 3-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 13.2 (2'-CH_3), 25.9 (C-4'), 115.0 (C-2), 116.4 (C-5), 125.4 (C-6), 129.1 (C-1), 135.8 (C-2'), 142.8 (C-1'), 148.9 (C-4), 149.1 (C-3), 202.9 (C-3'), 56.5 (3-OCH_3); EI-MS (rel. int.) *m/z*: 206 $[M]^+$ (100), 159 (85), 103 (60) (calcd. for $C_{12}H_{14}O_3$).

Licarin A (10) – Yellow oil; $[\alpha]_D^{25}$ –28.2 (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} nm: 240, 279; IR (KBr) ν_{max} cm^{-1}: 3680, 3019, 1214, 1032, 748; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, d, J = 4 Hz, H-9), 1.88 (3H, d, J = 6.4 Hz, H-9'), 3.45 (1H, m, H-7), 5.11 (1H, d, J = 9.6 Hz, H-8), 6.11 (1H, m, H-8'), 6.37 (1H, d, J = 15.6 Hz, H-7'), 6.77 (1H, s, H-2), 6.79 (1H, s, H-6), 6.91 (1H, dd, J = 8.8 Hz, H-6'), 6.89 (1H, d, J = 8.8 Hz, H-5'), 6.98 (1H, s, H-2'), 5.63 (1H, br s, 8-OH), 3.89 (3H, s, 3-OCH_3), 3.90 (3H, s, 3'-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 17.7 (C-9), 18.6 (C-9'), 45.8 (C-7), 94 (C-8), 109.1 (C-5'), 109.4 (C-6), 113.5 (C-2), 114.2 (C-2'), 120.2 (C-6'), 123.7 (C-8'), 131.1 (C-7'), 132.3 (C-4), 132.4 (C-1'), 133.4 (C-

1), 144.3 (C-5), 145.9 (C-3'), 146.7 (C-4'), 146.9 (C-3), 56.1 (3'-OCH_3), 56.2 (3-OCH_3); HR-FAB-MS *m/z*: 327.1593 $[M - 2OH + H]^+$] (calcd for $C_{20}H_{23}O_4$; 327.1596).

Cytotoxicity Assay – The cancer cell lines were maintained in RPMI 1640 (HL-60), and/or DMEM (A549, MCF-7), 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 150.0 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 aerial part of *S. chinensis* was partitioned into *n*-hexane-, EtOAc-, and *n*-BuOH-soluble fractions and a H_2O layer. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of ten compounds (**1 - 10**) (Fig. 1). The structures of compounds were identified as 7-hydroxysauchinone (**1**) (Lim *et al*., 2012) sauchinone (**2**) (Sung and Kim, 2000), di-*O*-methyltetrahydrofuriguaiacin B (**3**) (Hwang *et al*., 2003-2), henricine (**4**) (Chang *et al*., 2009), saucerneol K (**5**) (Suri *et al*., 1981), *meso*-dihydroguaiaretic acid (**6**) (Kwon *et al*., 2008), (−)-guaiacin (**7**) (Kwon *et al*., 2008), (3*R*,4*S*)-4-(4-hydroxy-3-methoxyphenyl)-4-methoxy-3-methylbutan-2-one (**8**) (Li *et al*., 1986), (*E*)-7-(4-hydroxy-3-methoxyphenyl)-7-methylbut-8-en-9-one (**9**) (Chang *et al*., 2009) and licarin A (**10**) (Lee *et al*., 2004; Pereira *et al*., 2011) by comparing their physiochemical and spectroscopic data with those reported in the literature.

Compounds **1 - 10** 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 result in Table 1, (−)-guaiacin (**7**) showed the most potent inhibitory activity against HL-60 cancer cell line with IC_{50} value of 11.5 μM, following by licarin A (**10**), *meso*-dihydro-

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

Compounds	IC_{50} (μM)		
	A549	HL-60	MCF-7
4	> 30	22.4	> 30
6	25.0	18.9	> 30
7	> 30	11.5	> 30
9	> 30	24.7	> 30
10	> 30	18.1	22.2
Adriamycin[a]	2.5	0.3	3.8

[a] Used as positive control

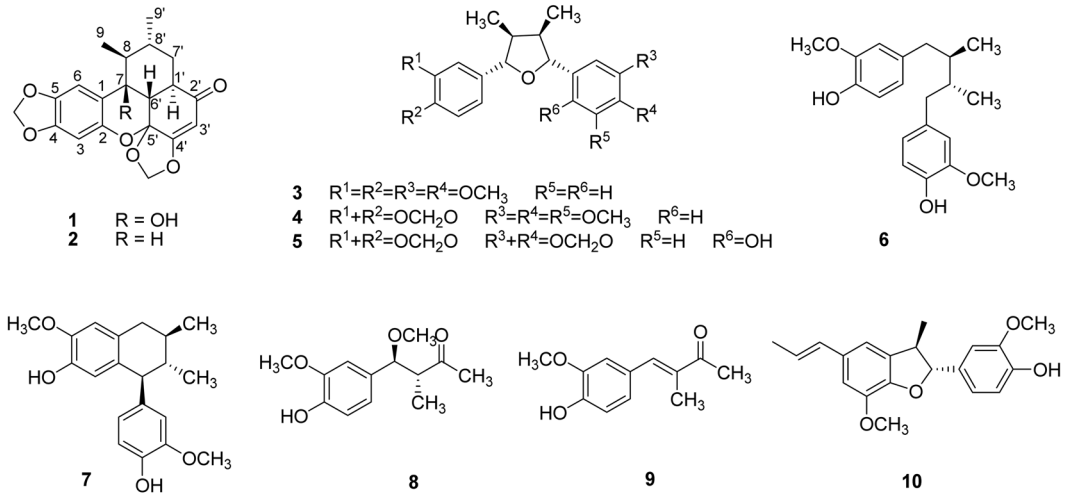

Fig. 1. Chemical structures of isolated compounds **1 - 10**.

guaiaretic acid (**6**), henricine (**4**), and (*E*)-7-(4-hydroxy-3-methoxyphenyl)-7-methylbut-8-en-9-one (**9**) with IC_{50} values of 18.1, 18.9, 22.4, and 24.7 μM, respectively. The other compounds showed weak cytotoxic activity with IC_{50} values over than 30 μM. In the case of A549 cancer cell line, *meso*-dihydroguaiaretic acid (**6**) showed the most potent cytotoxic activity with IC_{50} value of 25.0 μM. Except for the activity of licarin A with the IC_{50} value of 22.2 μM, the remains compounds showed weak cytotoxic activity against MCF-7 cancer cell line with IC_{50} values over than 30 μM.

Among the active compounds, *meso*-dihydroguaiaretic acid (**6**), which was isolated firstly from nutmeg, was evaluated on its anticancer activity in several cancer cell lines as A549 (human lung cancer), Colo205 (human colon cancer) and K562 (human leukemia). This compound showed an inhibitory effect on examined DNA-dimer complexes, including fos-jun, jun-jun and jun-GST-fused fos dimer. Also, the results suggested the possibility that anticancer activity of this compound may due to its inhibitory effect on the protein-DNA interaction (Park *et al*., 1998; Davis *et al*., 2009). In accordance with these results, this compound showed relaxation of super-coiled DNA to nicked DNA, it represented a structural type of DNA cleavage agent, and also showed significant cytotoxic effect on Hela cells (Chen *et al*., 2006). Compounds **6** and **7**, previously isolated from *Machilus wangchiana*, inhibited the release of β-glucuronidase in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activation factor (PAF), however, they did not showed cytotoxic effects on BGC-823 and A2780 cancer cells (Cheng *et al.,* 2009). Even though licarin A (**10**) was isolated and showed no cytotoxic activity against several cancer cell lines as P-388, KB16, A549 and HT-29 (Tsai *et al*., 2001), it should be noted that the cytotoxic activity of compounds **9** and **10** in HL-60 cancer cell were obtained for the first time. Recently, activity-guided fractionation of extract from *Saururus chinensis* led to isolation of several compounds including neolignans (manassantin A, manassantin B, (−)-saucerneol), flavonoids (quercetin-3-*O*-β-Dglucuronopyranoside, quercitrin, quercetin-3-*O*-(2"-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside, isoquercitrin) and aristolactams alkaloids (saurolactam, aristolactam AII, piperumbellactam B, aristolactam BII). These isolates were evaluated for their activities against 28 human cancer cell lines using an *in vitro* cell proliferation assay. However, only neolignans showed potent anti-proliferative activities against cervical (C33a) and lung (NCI-H460) cancer cells without any remarkable cytotoxic effects on human normal lung cells as a control (Lee *et al*., 2012). Our results in this study are in accordance with previous publication that lignan and neolignan structures from natural product showed their potential anticancer activity (Cuong *et al*., 2012). However, further works would be required to determine the details mechanism of action of these compounds.

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