Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Communication

Two flavonoids first isolated from the seed of *Syzygium nervosum* and preliminary study of their anticancer and anti-HIV-1 reverse transcriptase activities

Anuruk Chailungka[1], Thiti Junpirom[1], Wilart Pompimon[2], Narong Nuntasaen[3] and Puttinan Meepowpan [1, *]

[1] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[2] Laboratory of Natural Products, Centre for Innovation in Chemistry, Faculty of Science, Lampang Rajabhat University, Lampang 52100, Thailand
[3] The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok 10900, Thailand
* Corresponding author, e-mail: pmeepowpan@gmail.com

Received: 5 November 2015 / Accepted: 30 March 2017 / Published: 5 April 2017

Abstract: Two flavonoids, viz. 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) and hariganetin were first isolated from the dichloromethane extract of the seed of *Syzygium nervosum* and their biological activities against cancer cell lines and HIV-1 reverse transcriptase were preliminary studied. Both DMC and hariganetin show moderate activities against HIV-1 reverse transcriptase with 67.5% and 64.1% inhibition respectively.

Keywords: 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone, hariganetin, *Syzygium nervosum* , cytotoxicity, cancer cell lines, anti-HIV-1 reverse transcriptase

INTRODUCTION

The genus *Syzygium* (Myrtaceae) comprises approximately 1,200 species and is widely distributed in Africa, extending east to the Hawaiian Islands, and from India and China southwards to southern Australia and New Zealand. The centres of diversity are Southern Asia, South-east Asia, Malaysia, Australia and New Caledonia [1-4]. Many phytochemical substances from this genus have been isolated, e.g. terpenoids [5-7], triterpenes [8-11], phenylpropanoids [12-14], a macrocyclic ellagitannin [15], phenolic compounds [8, 16, 17], flavonoid glycosides [8, 17, 18], chalcones [11, 17, 19-21], flavanones [17, 19, 20], tannins [8] and lignins [8]. Some isolated compounds have been known to exhibit interesting biological properties including anti-HIV [6, 7], antibacterial [9], antimutagenic [12], antifungal [14], antitermitic [14], antioxidant [15-17],

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

anticancer [21] and cytotoxic [17] activities.

Syzygium nervosum A. Cunn. ex DC. is locally called Ma-kiang in northern Thailand. The old scientific name of the plant is *Cleistocalyx nervosum* var. *paniala*. It grows in scattered locations in villages of the northern provinces of Thailand such as Chiang Mai, Lumpun, Lumpang and Mae Hong Son [22]. Its edible fruit is sour and slightly astringent, and its rich purplish red colour is characteristic of a high level of anthocyanins [23]. The major active compounds from this plant have been identified as hydrolysable tannins and their derivatives, i.e. caffeoylquinic acid, gallic acid, ellagic acid and methoxymethyl gallate [24]. A previous study has shown that the ethanol extract from its fruit has stimulating activity on human lymphocytes and could be clinically useful for modulating the immune system of the body [25]. Its flesh and seed contain a high level of polyphenols and flavonoids [26, 27]. These compounds have antioxidant [24, 26, 28], anticarcinogenic [26, 29, 30] and antimutagenic [31] properties. Furthermore, the cold methanol extract of mature leaves shows the highest total phenolic and flavonoid contents and gives high free radical scavenging, lipid peroxidation inhibiting and tyrosinase inhibiting activities [32]. However, there are few reports focusing on their biological activities such as cytotoxicity against cancer cell lines and inhibitory effect on HIV-1 reverse transcriptase (RT).

Therefore, we report herein the isolation, chemical characterisation and bioactivities of two known flavonoids: 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (DMC) [11, 19, 20] and (2*S*)-4-hydroxy-6,6,8-trimethyl-2-phenyl-2*H*-1-benzopyran-5,7-(3*H*,6*H*)-dione (hariganetin) [33-35], first isolated from the seed of *S. nervosum*. Preliminary cytotoxicity assays against P-388, KB, HT29, MCF-7, A549, ASK, Hek293 cancer cell lines and inhibitory effects on HIV-1 RT are also reported.

MATERIALS AND METHODS

General

Melting points were determined by a Gallenkamp Electrothermal apparatus and were uncorrected. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ on a Bruker DRX 400 spectrometer. Chemical shifts are given in δ (ppm) downfield from tetramethylsilane (TMS) and coupling constants (*J* values) in Hz. Peak multiplicities are indicated as follows: *s* (singlet), *d* (doublet), *dd* (doublet of doublet) and *m* (multiplet). Infrared spectra were recorded on an FT-IR model TENSOR 27 (Bruker) spectrometer and absorption frequencies were reported in reciprocal centimeters (cm^{-1}). Mass spectra (EI-MS) were performed with a GC/MS Agilent technologies system 6850 II/5973 using ionisation energy of 70 eV. High-resolution mass spectrometry (HRMS, electrospray ionisation mode) was measured on a Q-TOF-2™ (Waters) spectrometer. Flash column chromatography was performed employing Merck silica gel 60 and Merck silica gel 60H. Thin layer chromatography (TLC) was performed with Merck silica gel 60 F_{254} aluminium plates. All solvents used for extraction and isolation were distilled at their boiling point ranges prior to use.

Plant Material and Extraction

Seeds of *S. nervosum* were collected in July-August 2009 from Lampang province, Thailand. The voucher specimen of the plant (BKF no. 187213) was deposited at the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

Air dried seeds (3,216.83 g) were extracted with 10 L of dichloromethane (2 x 3 days), followed by filtration. The filtrates were combined and evaporated *in vacuo* to give a dichloromethane extract (97.47 g).

Isolation

The isolation of compounds **1** and **2** was accomplished by using silica gel column chromatography with gradient elution. The dichloromethane extract (97.47 g) was fractionated by hexane-dichloromethane, dichloromethane-ethyl acetate and ethyl acetate-methanol respectively. All fractions were combined based on TLC profiles and evaporated to dryness *in vacuo* to give six fractions: F1-F6. F3 was further fractionated by repeated silica gel column chromatography into two subfractions to afford, after recrystallisation from CH_2Cl_2/hexane and EtOAc/hexane, compounds **1** (4.32 g) and **2** (1.18 g) respectively. In addition, **1** (3.48 g) and **2** (4.72 g) were also obtained from similar column chromatography of F4. The structure elucidation of **1** and **2** was achieved through comparison of their spectral data with previously reported data. The experimental data for the isolated compounds **1** and **2** are as follows.

Compound **1**: Orange solid, mp 120.8-122.3°C; EI-MS *m/z*: 298 $[M]^+$; HRMS (ESI) *m/z*: 321.1104, calcd 321.1103 for $C_{18}H_{18}O_4Na$ $[M+Na]^+$; IR (CH_2Cl_2) ($\nu_{max,}$ cm^{-1}): 3444, 2935, 1628, 1554, 1165; ^1H NMR (400 MHz, δ, ppm, *J*/Hz): 2.14 (3H, *s*, 5′-C$\underline{H}_3$), 2.16 (3H, *s*, 3′-C$\underline{H}_3$), 3.66 (3H, *s*, 6′-OC$\underline{H}_3$), 5.38 (1H, *s*, 4′-O$\underline{H}$), 7.41 (3H, *m*, H-3,4,5), 7.64 (2H, *m*, H-2,6), 7.84 (1H, *d*, *J* = 15.7 Hz, H_β), 7.99 (1H, *d*, *J* = 15.7 Hz, H_α), 13.69 (1H, *s*, 2′-O$\underline{H}$); ^{13}C NMR (100 MHz, δ, ppm): 7.6 (5′-$\underline{C}H_3$), 8.2 (3′-$\underline{C}H_3$), 62.3 (6′-O$\underline{C}H_3$), 106.6 (C-1′), 109.0 (C-3′), 109.0 (C-5′), 126.7 (C_α), 128.4 (C-5), 128.4 (C-3), 128.9 (C-6), 128.9 (C-2), 130.2 (C-4), 135.3 (C-1), 142.9 (C_β), 158.8 (C-6′), 159.3 (C-4′), 162.0 (C-2′), 193.4 (C=O).

Compound **2**: Orange solid, mp 140.4-141.5°C; EI-MS *m/z*: 298 $[M]^+$; HRMS (ESI) *m/z*: 321.1102, calcd 321.1103 for $C_{18}H_{18}O_4Na$ $[M+Na]^+$; IR (CH_2Cl_2) ($\nu_{max,}$ cm^{-1}): 3450, 2974, 1649, 1619, 1499, 1055; ^1H NMR (400 MHz, δ, ppm, *J*/Hz): 1.40 (3H, *s*, 6-C$\underline{H}_3$), 1.42 (3H, *s*, 6-C$\underline{H}_3$), 1.86 (3H, *s*, 8-C$\underline{H}_3$), 2.92 (1H, *dd*, *J* = 17.9, 3.7 Hz, H-3a), 3.03 (1H, *dd*, *J* = 17.9, 10.8 Hz, H-3b), 5.32 (1H, *dd*, *J* = 10.8, 3.7 Hz, H-2), 7.37-7.48 (5H, *m*, H-2′,3′,4′,5′,6′), 15.80 (1H, *s*, 4-O$\underline{H}$); ^{13}C NMR (100 MHz, δ, ppm): 7.9 (8-$\underline{C}H_3$), 23.1 (6-$\underline{C}H_3$), 25.5 (6-$\underline{C}H_3$), 38.2 (C-3), 52.3 (C-6), 76.0 (C-2), 101.4 (C-10), 107.3 (C-8), 125.8 (C-2′,6′), 128.9 (C-3′,4′,5′), 138.0 (C-1′), 161.2 (C-9), 182.8 (C-4), 198.0 (C-7), 201.7 (C-5).

Evaluation of Cytotoxic Activity

The isolated compounds were submitted to preliminary cytotoxicity assays against cancer cell lines at the cytotoxicity test Service Centre at the Department of Microbiology, Mahidol University, Thailand. The cytotoxic activities of extracts and compounds were determined using the standard sulforhodamine B (SRB) assay in 96-well microtiter plates [36, 37]. Ellipticine was used as a positive control as it directly interacts with DNA, which is the ultimate target of cancer chemotherapy [38, 39]. Altogether, seven cell lines were employed: P-388 (mouse lymphoid neoplasma), KB (human epidermoid carcinoma in the mouth), HT29 (human colon cancer), MCF-7 (human breast cancer), Lu-1 (human lung cancer), A549 (human lung cancer) and ASK (rat glioma), together with Hek 293 (noncancerous human embryonic kidney cell). The cytotoxic activity is expressed as 50% effective dose (ED_{50}).

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

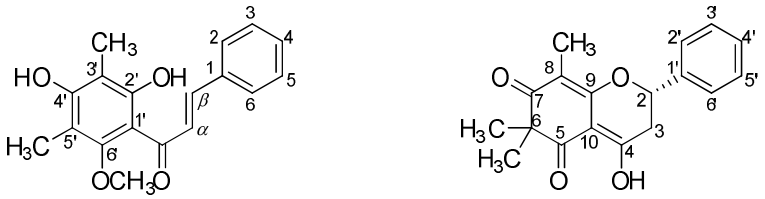
Anti-HIV-1 RT Assay

The isolated compounds were submitted to preliminary screening of inhibitory effect on HIV-1 RT at the cytotoxicity test Service Centre at the Department of Microbiology, Mahidol University, Thailand. The compounds were dissolved in DMSO at the concentration of 20 mg/mL and processed further to remove tannin. The assay was carried out in duplicate in a 96-well microtiter plate using the tannin-free supernatant of each compound as previously described [40]. An appropriate amount of HIV-1 RT (Amersham Pharmacia Biotech Asia Pacific Ltd., Hong Kong) was employed and standardised with fagaronine chloride. This compound and nevirapine were used as positive controls, while DMSO was used as a negative control. The test compounds were prescreened at 200 μg/mL. The results from duplicate wells were averaged and the percentage of inhibition was calculated.

RESULTS AND DISCUSSION

Structure Elucidation

The results of analysis were compared with spectral data from previous reports [11, 19, 20, 33-35] and the structures of **1** and **2** were established as 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) and (2*S*)-4-hydroxy-6,6,8-trimethyl-2-phenyl-2*H*-1-benzopyran-5,7-(3*H*,6*H*)-dione (hariganetin) respectively (Figure 1).



1 **2**

Figure 1. Structures of DMC (**1**) and hariganetin (**2**)

Compound **1** (orange solid) has a melting point of 120.8-122.3°C which is comparable to that reported in the literature, i.e. 125-126°C [19, 20]. The HRMS analysis shows $[M+Na]^+$ ion at *m/z* 321.1104, indicating a molecular formula of $C_{18}H_{18}O_4Na$ (calcd: 321.1103). The EI-MS exhibits a molecular ion peak at *m/z* 298 $[M^+]$, corresponding to a molecular formula of $C_{18}H_{18}O_4$ and fragmentation ions are found at *m/z* 221, 194, 166, 103 and 77, which is identical with the literature [11, 19, 20]. The IR spectrum shows a characteristic C=O stretching band of a conjugated carbonyl system at 1628 cm^{-1}. The absorption band at 3444 cm^{-1} is assigned to a free hydroxyl as well as an H-bonded hydroxyl group. The ^1H NMR (400 MHz) spectrum of compound **1** (Table 1) exhibits signals for a monosubstituted phenyl group at δ 7.41 and 7.64, a *trans*-disubstituted double bond at δ 7.84 and 7.99 (J_{AB} = 15.7 Hz) and two benzylic methyl groups at δ 2.14 and 2.16 ppm. Furthermore, the spectrum reveals the presence of three singlets for dihydroxyl and methoxyl groups at δ 13.69 (2′-OH), 5.38 (4′-OH) and 3.66 (6′-OCH_3) ppm.

The complete structure of **1** was determined by analysing the 2D-NMR data including heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra. The HMQC spectrum allows us to connect the protons and carbons as shown in Table 1. The HMBC spectrum (Table 2) shows correlation of the proton signal at δ 7.84 (*d*, *J* = 15.7

Hz, H_β) with carbon signals at δ 135.3 (C-1), 128.9 (C-2,6), 126.7 (C_α) and 193.4 (C=O). The proton signal at δ 7.99 (*d*, *J* = 15.7 Hz, H_α) correlates with carbon signals at δ 135.3 (C-1), 142.9 (C_β), 193.4 (C=O) and 106.6 (C-1′). The proton signal of the methyl group at δ 2.14 (*s*, 3′-C$\underline{H}_3$) shows correlation with the carbon signals at δ 162.0 (C-2′), 109.0 (C-3′) and 159.3 (C-4′). The signal at δ 2.16 (*s*, 5′-C$\underline{H}_3$) shows correlation with the carbon signals at δ 159.3 (C-4′), 109.0 (C-5′) and 158.8 (C-6′), and the proton signal of the methoxy group at 3.66 (*s*, 6′-OC$\underline{H}_3$) correlates with the carbon signal at δ 158.8 (C-6′). In addition, the spectroscopic data were almost identical to those reported in the literature for this compound [11, 19, 20].

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra in $CDCl_3$ for isolated flavonoids **1** and **2**

Position	2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (1)		Position	(2*S*)-4-Hydroxy-6,6,8-trimethyl-2-phenyl-2*H*-1-benzopyran-5,7-(3*H*,6*H*)-dione (2)	
	*δ^1H (*J* Hz)	δ^{13}C (DEPT)		*δ^1H (*J* Hz)	δ^{13}C (DEPT)
1	-	135.3 (C)	2	5.32 *dd* (10.8, 3.7)	76.0 (CH)
2	7.64 *m*	128.9 (CH)	3	2.92 *dd* (17.9, 3.7)	38.2 (CH_2)
3		128.4 (CH)		3.03 *dd* (17.9, 10.8)	
4	7.41 *m*	130.2 (CH)	4	-	182.8 (C)
5		128.4 (CH)	5	-	201.7 (C)
6	7.64 *m*	128.9 (CH)	6	-	52.3 (C)
β	7.84 *d* (15.7)	142.9 (CH)	7	-	198.0 (C)
α	7.99 *d* (15.7)	126.7 (CH)	8	-	107.3 (C)
CO	-	193.4 (C)	9	-	161.2 (C)
1′	-	106.6 (C)	10	-	101.4 (C)
2′	-	162.0 (C)	1′	-	138.0 (C)
3′	-	109.0 (C)	2′		125.8 (CH)
4′	-	159.3 (C)	3′		128.9 (CH)
5′	-	109.0 (C)	4′	7.37-7.48 *m*	128.9 (CH)
6′	-	158.8 (C)	5′		128.9 (CH)
2′-O$\underline{H}$	13.69 *s*	-	6′		125.8 (CH)
3′-$\underline{C}H_3$	2.14 *s*	8.2 (CH_3)	4-OH	15.80 *s*	-
4′-O$\underline{H}$	5.38 *s*	-	6-CH_3	1.40 *s*	23.1 (CH_3)
5′-$\underline{C}H_3$	2.16 *s*	7.6 (CH_3)	6-CH_3	1.42 *s*	25.5 (CH_3)
6′-O$\underline{C}H_3$	3.66 *s*	62.3 (CH_3)	8-CH_3	1.86 *s*	7.9 (CH_3)

* δ in ppm from TMS [coupling constants (*J*) in Hz are given in parentheses.]

Table 2. ^{1}H-^{13}C and ^{1}H-^{1}H correlations for isolated flavonoids **1** and **2**

Position H	2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (1)		Position H	(2*S*)-4-Hydroxy-6,6,8-trimethyl-2-phenyl-2*H*-1-benzopyran-5,7-(3*H*,6*H*)-dione (2)	
	HMBC Correlation	COSY Correlation		HMBC Correlation	COSY Correlation
2	C-3, 4, *β*	H-3	2	C-3, 4, 1′, 2′, 6′	H-3
3	C-1, 2, 5	H-2, 4	3	C-2, 4, 10, 1′	H-2
4	C-2, 3, 5, 6	H-3, 5	2′	C-2, 1′, 3′, 4′, 6′	H-3′
5	C-1, 3, 6	H-4, 6	3′	C-1′, 2′, 4′, 5′	H-2′, 4′
6	C-2, 4, 5	H-5	4′	C-2′, 3′, 5′, 6′	H-3′, 5′
β	C-1, 2, 6, *α*, CO	H-*α*	5′	C-1′, 3′, 4′, 6′	H-4′, 6′
α	C-1, 1′, *β*, CO	H-*β*	6-C$\underline{H}_3$	C-5, 6, 7	-
3′-C$\underline{H}_3$	C-2′, 3′, 4′	-	6-C$\underline{H}_3$	C-5, 6, 7	-
5′-C$\underline{H}_3$	C-4′, 5′, 6′	-	8-C$\underline{H}_3$	C-7, 8, 9	-
6′-OC$\underline{H}_3$	C-6′	-			

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

Compound **2**, isolated also as an orange solid, has a melting point of 140.4-141.5°C. It was determined as $C_{18}H_{18}O_4Na$ by HRMS, showing the $[M+Na]^+$ ion at *m/z* 321.1102 (calcd: 321.1103). The EI-MS exhibits a molecular ion peak at *m/z* 298 $[M]^+$, corresponding to a molecular formula of $C_{18}H_{18}O_4$. The IR spectrum exhibits the C=O stretching of two carbonyl groups at 1619 and 1649 cm^{-1} and the carbonyl conjugated C=C stretching at 1449 cm^{-1}. The absorption bands at 3450 and 1168 cm^{-1} are assigned to the O-H and C-O stretching respectively. The ^1H NMR spectrum (Table 1) shows three methyl protons appearing as singlets at δ 1.40, 1.42 and 1.86 ppm (9H, *s*, 6,6,8-C<u>H</u>$_3$ respectively) and one hydroxyl proton at δ 15.80 ppm (*s*, 4-O<u>H</u>), hydrogen-bonded to the carbonyl group. The protons at the 3-position appear as a doublet-of-doublet at δ 2.92 (J = 17.9, 3.7 Hz) and 3.03 (J = 17.9, 10.8 Hz) and the oxymethine proton H-2 shows a doublet-of-doublet signal at δ 5.32 (J = 10.8, 3.7 Hz) ppm. The ^{13}C NMR spectrum displays several characteristic signals: the C-4 carbon with a hydroxyl group shows a signal at δ 182.8 ppm; the two carbonyl carbons C-5 and C-7 show peaks at δ 201.7 and 198.0 ppm respectively; and the endocyclic double bond at C-9 shows a unique signal at δ 161.2 ppm.

Furthermore, 2D-NMR spectra, HMQC and HMBC were also recorded for compound **2**, as shown in Table 2, to confirm the structure. The HMQC data are used to explain the correlation between ^1H and ^{13}C NMR spectra. They display correlations such as the H-2 proton being coupled by C-2 carbon, the H-3 proton being associated with C-3 carbon, and the three methyl protons showing correlations with 8-CH_3 and 6-$(CH_3)_2$ carbons at δ 7.9, 23.1 and 25.5 ppm respectively.

The HMBC spectrum shows correlations of the proton signals at δ 2.92 and 3.03 of the methylene protons at H-3 with the carbon signals at δ 76.0 (C-2), 182.8 (C-4), 101.4 (C-10) and 138.0 (C-1′); the proton signal at δ 5.32 (H-2) correlates with the carbon signals at δ 38.2 (C-3), 182.8 (C-4), 138.0 (C-1′) and 125.8 (C-2′, 6′); the proton signal at δ 1.86 (*s*, 8-C<u>H</u>$_3$) shows association with the carbon signals at δ 198.0 (C-7), 107.3 (C-8) and 161.2 (C-9); and the proton signals of the dimethyl group at δ 1.40 and 1.42 (*s*, 6-C<u>H</u>$_3$) show correlation with the carbon signals at δ 201.7 (C-5), 52.3 (C-6) and 198.0 (C-7). These spectral analyses were compared to the literature [33-35] and the structure is established to be hariganetin (**2**).

Biological Activities

DMC (**1**) has been reported regarding its biological properties such as antibiotic [41], antitumour [42-44], anticancer [21], antiprotozoal [45] and cytotoxic [17, 46, 47] activities. In addition, it shows an inhibitory effect on the viral neuraminidases from two influenza viral strains, H1N1 and H9N2 [48]. In our investigation the two isolated flavonoids were preliminarily evaluated for anticancer and and anti-HIV-1 RT activities (Table 3). DMC (**1**) shows recordable cytotoxicity to P-388, KB, HT29, MCF-7, A549, ASK and Hek293 cell lines, while hariganetin (**2**) is inactive. However, both compounds (**1** and **2**) exhibit an anti-HIV-1 RT activity, with an inhibition of 67.5% and 64.1% respectively.

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

Table 3. Cytotoxicity and anti-HIV-1 RT activities of isolated flavonoids

Compound	Cytotoxicity (ED_{50}, μg/mL)							HIV-1 RT assay % Inhibition at 200 μg/mL
	P-388	KB	HT29	MCF-7	A549	ASK	HeK293	
DMC (**1**)	10.31	15.90	NR	14.51	13.04	9.00	2.39	67.5
Hariganetin (**2**)	NR	NR	NR	NR	NR	NR	NR	64.1
Ellipticine (Positive control)	0.56	0.53	0.61	0.51	0.48	0.38	0.49	NT

Notes: ED_{50} less than 20 μg mL^{-1} for extract and less than 4 μg mL^{-1} for pure compound are considered active. P-388 = murine lymphocytic leukemia, KB = human oral nasopharyngal carcinoma, HT29 = human colon cancer, MCF-7 = human breast cancer, A549 = human lung cancer, ASK = rat glioma cell, HeK293 = noncancerous human embryonic kidney cell. Ellipticine, an anticancer drug, was used as a positive control in the cytotoxicity test. NR = no response (ED_{50}>20 μg mL^{-1}), NT = not tested.

ACKNOWLEDGEMENTS

This research was supported by Chiang Mai University Mid-Career Research Fellowship Programme and Centre of Excellence for Innovation in Chemistry (PERCH-CIC). The Research Administration Centres of Chiang Mai University and North-Chiang Mai University are also thanked for financial support.

REFERENCES

1. L. A. Craven, "Unravelling knots or plaiting rope: What are the major taxonomic strands in *Syzygium* sens.lat. (Myrtaceae) and what should be done with them?", Proceedings of 4th Flora Malesiana Symposium, **2001**, Kuala Lumpur, Malaysia, pp.75-85.
2. L. A. Craven and E. Biffin, "An infrageneric classification of *Syzygium* (Myrtaceae)", *Blumea*, **2010**, *55*, 94-99.
3. S. H. Tuiwawa, L. A. Craven, C. Sam and M. D. Crisp, "The genus *Syzygium* (*Myrtaceae*) in Vanuatu", *Blumea*, **2013**, *58*, 53-67.
4. J. A. N. Parnell, L. A. Craven and E. Biffin, "Matters of scale: Dealing with one of the largest genera of angiosperms", in "Reconstructing the Tree of Life: Taxonomy and Systematics of Species Rich Taxa" (Ed. T. R. Hodkinson and J. A. N. Parnell), CRC Press, Boca Raton, **2007**, Ch.16.
5. C.-W. Chang, T.-S. Wu, Y.-S. Hsieh, S.-C. Kuo and P.-D. L. Chao, "Terpenoids of *Syzygium formosanum*", *J. Nat. Prod.*, **1999**, *62*, 327-328.
6. Y. Kashiwada, H. K. Wang, T. Nagao, S. Kitanaka, I. Yasuda, T. Fujioka, T. Yamagishi, L. M. Cosentino, M. Kozuka, H. Okabe, Y. Ikeshiro, C. Q. Hu, E. Yeh and K. H. Lee, "Anti-AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids", *J. Nat. Prod.*, **1998**, *61*, 1090-1095.
7. Y. Kashiwada, T. Nagao, A. Hashimoto, Y. Ikeshiro, H. Okabe, L. M. Cosentino and K. H. Lee, "Anti-AIDS agents 38. Anti-HIV activity of 3-*O*-acyl ursolic acid derivatives", *J. Nat. Prod.*, **2000**, *63*, 1619-1622.
8. L.-W. Tian, M. Xu, D. Wang, H.-T. Zhu, C.-R. Yang and Y.-J. Zhang, "Phenolic constituents from the leaves of *Syzygium forrestii* Merr. and Perry", *Biochem. Syst. Ecol.*, **2011**, *39*, 156-158.

9. J. D. Djoukeng, E. Abou-Mansour, R. Tabacchi, A. L. Tapondjou, H. Bouda and D. Lontsi, "Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae)", *J. Ethnopharmacol.*, **2005**, *101*, 283-286.
10. Q.-H. Han, D.-M. Wang, Z.-B. Cheng, X. Yang, X.-J. Xu, J. Wang and S. Yin, "Chemical constituents from the leaves and twigs of *Syzygium tetragonum* Wall", *Biochem. Syst. Ecol.*, **2012**, *41*, 3-5.
11. R. Srivastava, A. K. Shaw and D. K. Kulshreshtha, "Triterpenoids and chalcone from *Syzygium samarangense*", *Phytochem.*, **1995**, *38*, 687-689.
12. M. Miyazawa and M. Hisama, "Antimutagenic activity of phenylpropanoids from clove (*Syzygium aromaticum*)", *J. Agric. Food Chem.*, **2003**, *51*, 6413-6422.
13. M. Miyazawa and M. Hisama, "Suppression of chemical mutagen-induced SOS response by alkylphenols from clove (*Syzygium aromaticum*) in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test", *J. Agric. Food Chem.*, **2001**, *49*, 4019-4025.
14. Y. Xie, Z. Yang, D. Cao, F. Rong, H. Ding and D. Zhang, "Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (clove)", *Ind. Crops Prod.*, **2015**, *77*, 780-786.
15. Y. Nomi, S. Shimizu, Y. Sone, M. T. Tuyet, T. P. Gia, M. Kamiyama, T. Shibamoto, K. Shindo and Y. Otsuka, "Isolation and antioxidant activity of zeylaniin A, a new macrocyclic ellagitannin from *Syzygium zeylanicum* leaves", *J. Agric. Food Chem.*, **2012**, *60*, 10263-10269.
16. A. Gordon, E. Jungfer, B. A. da Silva, J. G. Maia and F. Marx, "Phenolic constituents and antioxidant capacity of four underutilized fruits from the Amazon region", *J. Agric. Food Chem.*, **2011**, *59*, 7688-7699.
17. M. J. Simirgiotis, S. Adachi, S. To, H. Yang, K. A. Reynertson, M. J. Basile, R. R. Gil, I. B. Weinstein and E. J. Kennelly, "Cytotoxic chalcones and antioxidants from the fruits of a *Syzygium samarangense* (Wax Jambu)", *Food. Chem.*, **2008**, *107*, 813-819.
18. M. N. Samy, S. Sugimoto, K. Matsunami, H. Otsuka and M. S. Kamel, "One new flavonoid xyloside and one new natural triterpene rhamnoside from the leaves of *Syzygium grande*", *Phytochem. Lett.*, **2014**, *10*, 86-90.
19. E. C. Amor, I. M. Villasenor, A. Yasin and M. I. Choudhary, "Prolyl endopeptidase inhibitors from *Syzygium samarangense* (Blume) Merr. & L. M. Perry", *Z. Naturforsch. C.*, **2004**, *59*, 86-92.
20. E. C. Amor, I. M. Villasenor, S. A. Nawaz, M. S. Hussain and M. I. Choudhary, "A dihydrochalcone from *Syzygium samaragense* with anticholinesterase activity", *Philipp. J. Sci.*, **2005**, *134*, 105-111.
21. A. H. Memon, Z. Ismail, A. F. A. Aisha, F. S. R. Al-Suede, M. S. R. Hamil, S. Hashim, M. A. A. Saeed, M. Laghari and A. M. S. A. Majid, "Isolation, characterization, crystal structure elucidation, and anticancer study of dimethyl cardamonin, isolated from *Syzygium campanulatum* Korth", *Evidence-Based Complement. Alternat. Med.*, **2014**, *2014*, Art. ID 470179.
22. S. Thongma, "Ma-kiang", Lampang Agricultural Research and Training Center, Bangkok, **2002**, pp.66-96.
23. C. Jansom, S. Bhamarapravati and A. Itharat, "Major anthocyanin from ripe berries of *Cleistocalyx nervosum* var. *paniala*", *Thammasat Med. J.*, **2008**, *8*, 364-370.
24. N. Nuengchamnong and K. Ingkaninan, "On-line characterization of phenolic antioxidants in fruit wines from family myrtaceae by liquid chromatography combined with electrospray

ionization tandem mass spectrometry and radical scavenging detection", *LWT-Food. Sci. Technol.*, **2009**, *42*, 297-302.

25. B. Sriwanthana, W. Treesangsri, B. Boriboontrakul, S. Niumsakul and P. Chavalittumrong, "*In vitro* effects of Thai medicinal plants on human lymphocyte activity", *Songklanakarin J. Sci. Technol.,* **2007**, *29*, 17-28.
26. S. Chaisawadi and W. Methawiriyasilp, "Clean production of commercial 'Makiang' juice processing for medicinal herbs and health benefits", *Acta Hortic.*, **2008**, *786*, 201-208.
27. N. Narkprasom, K. Narkprasom and U. Upara, "Optimization of total phenolic from *Cleistocalyx nervosum* by microwave-assisted extraction", *Am. J. Eng. Appl. Sci.*, **2015**, *8*, 302-309.
28. W. Poontawee, S. Natakankitkul and O. Wongmekiat, "Protective effect of *Cleistocalyx nervosum* var. *paniala* fruit extract against oxidative renal damage caused by cadmium", *Molecules*, **2016**, *21*, 133.
29. W. Inboot, S. Taya, A. Chailungka, P. Meepowpan and R. Wongpoomchai, "Genotoxicity and antigenotoxicity of the methanol extract of *Cleistocalyx nervosum* var. *paniala* seed using a Salmonnella mutation assay and rat liver micronucleus tests", *Mol. Cell. Toxicol.*, **2012**, *8*, 19-24.
30. S. Taya, C. Punvittayagul, W. Inboot, S. Fukushima and R. Wongpoomchai, "*Cleistocalyx nervosum* extract ameliorates chemical-induced oxidative stress in early stages of rat hepatocarcinogenesis", *Asian Pac. J. Cancer Prev.*, **2014**, *15*, 2825-2830.
31. S. Charoensin, S. Taya, S. Wongpornchai and R. Wongpoomchai, "Assessment of genotoxicity and antigenotoxicity of an aqueous extract of *Cleistocalyx nervosum* var. *Paniala* in *in vitro* and *in vivo* models", *Interdiscip. Toxicol.*, **2012**, *5*, 201-206.
32. J. Manosroi, C. Chankhampan, K. Kumguan, W. Manosroi and A. Manosroi, "*In vitro* anti-aging activities of extracts from leaves of Ma Kiang (*Cleistocalyx nervosum* var. *paniala*)", *Pharm. Biol.*, **2015**, *53*, 862-869.
33. H. Wada, N. Tanaka, T. Murakami, T. Uchida, K. Kozawa, Y. Saiki and C. M. Chen, "Chemical and chemotaxonomical studies of Filices. LXXVI. An unusual flavanone derivative from *Wagneriopteris japonica* Loeve *et* Loeve", *Yakuga. Zasshi*., **1988**, *108*, 740-744.
34. N. T. Nguyen, V. C. Pham, M. Litaudon, F. Gueritte, B. Bodo, V. T. Nguyen and V. H. Nguyen, "Novel cyclopeptide and unique flavone from *Desmos rostrata.* Total synthesis of desmorostratone", *Tetrahedron*, **2009**, *65*, 7171-7176.
35. J. Fang, C. Paetz and B. Schneider, "C-methylated flavanones and dihydrochalcones from *Myrica gale* seeds", *Biochem. Syst. Ecol.*, **2011**, *39*, 68-70.
36. K. Likhitwitayawuid, C. K. Angerhofer, G. A. Cordell, J. M. Pezzuto and N. Ruangrungsi, "Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Stephania erecta*", *J. Nat. Prod.*, **1993**, *56*, 30-38.
37. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, "New colorimetric cytotoxicity assay for anticancer-drug screening", *J. Natl. Cancer Inst*., **1990**, *82*, 1107-1112.
38. M. Stiborová, J. Sejbal, L. Bořek-Dohalská, D. Aimová, J. Poljaková, K. Forsterová, M. Rupertová, J. Wiesner, J. Hudeček, M. Wiessler and E. Frei, "The anticancer drug ellipticine forms covalent DNA adducts, mediated by human cytochromes P450, through metabolism to 13-hydroxyellipticine and ellipticine N^2-oxide", *Cancer. Res.*, **2004**, *64*, 8374-8380.

Maejo Int. J. Sci. Technol. **2017**, *11*(01), 58-67

39. V. Vichai and K. Kirtikara, "Sulforhodamine B colorimetric assay for cytotoxicity screening", *Nat. Protoc.*, **2006**, *1*, 1112-1116.
40. G. T. Tan, J. M. Pezzuto, A. D. Kinghorn and S. H. Hughes, "Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase", *J. Nat. Prod.*, **1991**, *54*, 143-154.
41. G. Belofsky, D. Percivill, K. Lewis, G. P. Tegos and J. Ekart, "Phenolic metabolites of *Dalea versicolor* that enhance antibiotic activity against model pathogenic bacteria", *J. Nat. Prod.*, **2004**, *67*, 481-484.
42. C.-L. Ye, J.-W. Liu, D.-Z. Wei, Y.-H. Lu and F. Qian, "*In vitro* anti-tumor activity of 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone against six established human cancer cell lines", *Pharmacol. Res.*, **2004**, *50*, 505-510.
43. C.-L. Ye, J.-W. Liu, D.-Z. Wei, Y.-H. Lu and F. Qian, "*In vivo* antitumor activity by 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone in a solid human carcinoma xenograft model", *Cancer Chemother. Pharmacol.*, **2005**, *55*, 447-452.
44. X.-F. Zhu, B.-F. Xie, J.-M. Zhou, G.-K. Feng, Z.-C. Liu, X.-Y. Wei, F.-X. Zhang, M.-F. Liu and Y.-X. Zeng, "Blockade of vascular endothelial growth factor receptor signal pathway and antitumor activity of ON-III (2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone), a component from Chinese herbal medicine", *Mol. Pharmacol.*, **2005**, *67*, 1444-1450.
45. M. M. Salem and K. A. Werbovetz, "Antiprotozoal compounds from *Psorothamnus polydenius*", *J. Nat. Prod.*, **2005**, *68*, 108-111.
46. E. C. Amor, I. M. Villaseñor, R. Antemano, Z. Perveen, G. P. Concepcion and M. I. Choudhary, "Cytotoxic C-methylated chalcones from *Syzygium samarangense*", *Pharm. Biol.*, **2007**, *45*, 777-783.
47. C.-L. Ye and Y.-F. Lai, "2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone, from buds of *Cleistocalyx operculatus*, induces apoptosis in human hepatoma SMMC-7721 cells through a reactive oxygen species-dependent mechanism", *Cytotechnol.*, **2016**, *68*, 331-341.
48. T.-T. Dao, B.-T. Tung, P.-H. Nguyen, P.-T. Thuong, S.-S. Yoo, E.-H. Kim, S.-K. Kim and W.-K. Oh, "C-Methylated flavonoids from *Cleistocalyx operculatus* and their inhibitory effects on novel influenza A (H1N1) neuraminidase", *J. Nat. Prod.*, **2010**, *73*, 1636-1642.

© 2017 by Maejo University, San Sai, Chiang Mai, 50290 Thailand. Reproduction is permitted for noncommercial purposes.