Phytochemistry 80 (2012) 42–49

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Alkenylresorcinols and cytotoxic activity of the constituents isolated from *Labisia pumila*

Nabil Ali Al-Mekhlafi [a], Khozirah Shaari [a,b], Faridah Abas [a,c], Ralf Kneer [d], Ethel Jeyaseela Jeyaraj [e], Johnson Stanslas [e], Naoshi Yamamoto [f], Toshio Honda [f], Nordin H. Lajis [a,b,*]

[a] *Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[b] *Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[c] *Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[d] *Borneo Plant Technology Sdn. Bhd., Kuching, Sarawak, Malaysia*
[e] *Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*
[f] *Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa, Tokyo 142-8501, Japan*

ARTICLE INFO

Article history:
Received 4 April 2011
Received in revised form 20 February 2012
Available online 26 May 2012

Keywords:
Labisia pumila
Myrsinaceae
Cytotoxicity
1,4-Benzoquinone derivatives
Alkylresorcinol

ABSTRACT

Phytochemical investigation on the leaves of *Labisia pumila* (Myrsinaceae), an important medicinal herb in Malaysia, has led to the isolation of 1-*O*-methyl-6-acetoxy-5-(pentadec-10*Z*-enyl)resorcinol (**1**), labisiaquinone A (**2**) and labisiaquinone B (**3**). Along with these, 16 known compounds including 1-*O*-methyl-6-acetoxy-5-pentadecylresorcinol (**4**), 5-(pentadec-10*Z*-enyl)resorcinol (**5**), 5-(pentadecyl)resorcinol (**6**), (−)-loliolide (**7**), stigmasterol (**8**), 4-hydroxyphenylethylamine (**9**), 3,4,5-trihydroxybenzoic acid (**10**), 3,4-dihydroxybenzoic acid (**11**), (+)-catechin (**12**), (−)-epicatechin (**13**), kaempferol-3-*O*-α-rhamnopyranosyl-7-*O*-β-glycopyranoside (**14**), kaempferol-4′-*O*-β-glycopyranoside (**15**), quercetin-3-*O*-α-rhamnopyranoside (**16**), kaempferol-3-*O*-α-rhamnopyranoside (**17**), (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**18**) and stigmasterol-3-*O*-*β*-glycopyranoside (**19**) were also isolated. The structures of these compounds were established on the basis of 1D and 2D NMR spectroscopy techniques (^{1}H, ^{13}C, COSY, HSQC, NOESY and HMBC experiments), mass spectrometry and chemical derivatization. Among the constituents tested **1** and **4** exhibited strongest cytotoxic activity against the PC3, HCT116 and MCF-7 cell lines (IC_{50} values ⩽10 μM), and they showed selectivity towards the first two-cell lines relative to the last one.

1. Introduction

Labisia pumila is a small herbaceous plant, belonging to the family Myrsinaceae. It is widely distributed in the lowland and hilly rain forests of Malaysia, Thailand, Indochina, the Philippines and New Guinea (Jamia and Houghton, 2000; Ong, 2004; Stone, 1988; Wiart and Wong, 2002). Three varieties of *L. pumila* are available in Malaysia, classified as *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *Lanceolata* (Stone, 1988). All of these are collectively known as "Kacip Fatimah" among the local population. *L. pumila* var. *alata*, has long been used in traditional medicine by the indigenous communities in the Malay archipelago for the treating pre- and post-partum complications, menstrual disorders, in addition to being used as a health tonic to regain vigor. Other traditional uses include for the treatment of dysentery, rheumatism and anti-flatulence (Jaganath, 2000).

Despite its long use in traditional medicine, it is only recently that the herb was exploited commercially as health supplements. Nowadays, products in the forms of tablets, capsules, health drinks and tonics, derived from the plant extracts have become common in pharmacy and retail outlets (Latiff, 1997; Singh et al., 2009).

The traditional uses of *L. pumila* are associated mainly with estrogenic properties. This has been supported by several studies on its biological effects (Jamal et al., 1998). The species also showed antibacterial (Karimi et al., 2011), anti-inflammatory (Ibrahim et al., 1996; Rasadah et al., 2001) and antioxidant (Mohamad et al., 2009) properties. It has also been reported to reduce the risk of cardiovascular diseases (Mohamud and Nazaimoon, 2009) and protect skin cells from photo aging caused by UVB irradiation (Choi et al., 2010). Plants of the Myrsinaceae family have been known to exhibit a number of interesting pharmacological properties such as anti-inflammatory, antiviral, anti-tumor (Kobayashi and De Mejía, 2005), cytotoxic (Chang et al., 2009), and antileishmanial (Jimenez-Romero et al., 2007) activities. Investigations on a few species of the Myrsinaceae family have revealed the presence of various types of compounds including saponins, coumarins, quinines (Kobayashi and De Mejía, 2005), triterpenoid saponins

* Corresponding author. Current address: Scientific Chairs Unit, College of Medicine, Taibah University, P.O. Box 30001, Al-Madinah Al-Munawarah, Saudi Arabia. Tel.: +60 3 89468082; fax: +60 3 89468080.
E-mail address: nordinlajis@gmail.com (N.H. Lajis).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.04.008

(Tang et al., 2009), hydroxyalkenylresorcinols (Jimenez-Romero et al., 2007), alkenylphenols (Horgen et al., 1997), isoflavones (Kikuchi et al., 2009), dimeric lactones (Dat et al., 2007) and dimeric benzoquinone derivatives (Liu et al., 2009). However, very little is known about the phytochemistry of *L. pumila*. The medicinal importance of the species and the lack of information with regards to its chemical constituents prompted this investigation on the plant. Herein, identification of three new alkenylresorcinols, the occurrence of 16 other known compounds, and evaluation of the cytotoxic activity of some the isolated compounds are reported.

2. Results and discussion

The aqueous methanolic extract from the leaves of *Labisia* was sequentially partitioned from water into $CHCl_3$, EtOAc and *n*-BuOH fractions. Subsequent chromatographic purification of each fraction led to the isolation of three new compounds including an alkyl resorcinol (**1**) and two dimeric 1,4-benzoquinone derivatives (**2** and **3**), in addition to 16 other known compounds. The structures of these compounds were established mainly on the basis of analysis of NMR and mass spectroscopic data.

Compound **1** was obtained as colorless oil. The IR spectrum displayed absorptions at 3437 and at 1747 cm^{-1} indicating the presence of hydroxyl and ester carbonyl functionalities, respectively. The HRESI–MS of compound **1** exhibited a protonated molecular ion $[M+H]^+$ peak at *m/z* 391.2852 (calcd. for $C_{24}H_{38}O_4$+H, 391.2843), which corresponded to the molecular formula $C_{24}H_{38}O_4$ and six degrees of unsaturation. Analysis of the NMR spectroscopic data of **1** indicated the presence of a tetra-substituted aromatic ring with substituents being an alkenyl chain, a hydroxyl, a methoxyl and an acetyl groups. The ^{1}H NMR spectrum showed a pair of aromatic *meta*-coupled protons at δ 6.24 (H-2) and 6.18 (H-4), as well as proton signals belonging to a methoxyl group at δ 3.71, olefinic methines at δ 5.35 (overlapped, H-10′, H-11′), two allylic methylenes at δ 2.01 (overlapped, H-9′, H-12′), methylene at 1.50 (*m*, H-2′), eight other methylenes at δ 1.32–1.27 (overlapped), and a terminal methyl at δ 0.89 (*t*, H-15′). Analysis of ^{13}C NMR spectrum supported these deductions by the signals assignable to the tetra-substituted benzene ring carbons and to the C_{15} alkenyl chain (Table 1). Resonances for the methyl and carbonyl carbons of acetyl group was also observed at δ_C 20.7 (δ_H 2.33, 3H, *s*) and 170.3, respectively. The substitution pattern on the ring was further deduced based on HMBC correlations (Fig. 1). Correlations between methoxyl protons at (δ_H 3.71) with C-1 (δ_C 151.8) were observed, in addition to those of H-2 (δ_H 6.24) with C-6 (δ_C 131.7), C-1 (δ_C 151.8) and C-3 (δ_C 154.3), and between H-4 (δ_H 6.18) with C-6 (δ_C 131.7), C-2 (δ_C 98.4) and C-3 (δ_C 154.3), suggesting that the methoxyl and the hydroxyl groups are located at C-1 and C-3, respectively. The connectivity of the alkenyl chain to C-5 was established based on the key correlations observed between H-1′ (δ_H 2.39) and C-6 (δ_C 131.7), C-5 (δ_C 136.7), as well as C-4 (δ_C 107.9).

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compound **1** measured in $CDCl_3$.

Position	δ_H (*J* in Hz)	δ_C
1		151.8
2	6.24 (*d*, *J* = 2.5 Hz)	98.4
3		154.3
4	6.18 (*d*, *J* = 2.5 Hz)	107.9
5		136.7
6		131.7
1′	2.39 (*t*, *J* = 7.5 Hz)	32.2
2′	1.50 (*m*)	30.3
3′–8′	1.32–1.27 (*m*)	29.6–30.2
9′	2.01 (*m*)	27.2
10′	5.35 (*m*)	130.0
11′	5.35 (*m*)	130.0
12′	2.01 (*m*)	27.2
13′–14′	1.32–1.27 (*m*)	22.6
15′	0.89 (*t*, *J* = 7.0 Hz)	14.3
2-OCH_3	3.71 (*s*)	56.1
1-O$\underline{C}$O$\underline{CH}_3$	2.32 (*s*)	170.3
1-OCO$\underline{C}H_3$	–	20.7

The assignment of the double bond at C-10′ was based on the fragment ion peaks observed at *m/z* 348 $[M-C_3H_7-H]^+$ and 292 $[M-C_7H_{13}-H]^+$ resulting from the allylic cleavages (Suzuki et al., 1996) (Scheme. 1). This was further confirmed by ozonolysis (Barr et al., 1989) of the acetylated analogue (**1c**) which gave an aldehyde adduct (**1d**) exhibiting the protonated molecular ion peak $[M+H]^+$ at *m/z* 379.2119 (Scheme. 1). The configuration of the double bond was assigned as *Z* based on the ^{13}C NMR diagnostic chemical shift value of the allylic carbons (δ_C 27.2), which differed significantly from the reported chemical shift values of δ_C = 33 for the corresponding allylic carbons of *E* isomers (De Haan and Van de Ven, 1973; Liu et al., 2009). On the basis of these considerations, compound **1** was assigned as 1-*O*-methyl-6-acetoxy-5-(pentadec-10*Z*-enyl)resorcinol. Recently, the 8(*Z*) isomer of this compound was reported from *Ardisia brevicaulis*, also a member of the Myrsinaceae family (Bao et al., 2010).

Compounds **2** and **3** were isolated as red semi-solids, both having very similar UV, IR and NMR spectra. The UV and IR spectra were indicative of the presence of a phenol and 1,4-benzoquinone moieties, similar to those of belamcandaquinone found in *Ardisia* species (Liu et al., 2009).

The molecular formula of **2** was assigned $C_{43}H_{66}O_5$ as deduced from HR-ESI-MS, which exhibited a protonated molecular ion $[M+H]^+$ at *m/z* 663.4999 (calcd. for $C_{43}H_{66}O_5$+H, 663.4989), thus indicating 11 degrees of unsaturation. In the IR spectrum, absorption bands for hydroxyl was observed at 3283 cm^{-1}, for 1,4-quinone at 1679 and 1638 cm^{-1}, and for alkenyl at 1619 and 1601 cm^{-1}. The ^{1}HNMR spectrum exhibited resonances for two alkenyl side chains [δ_H 5.32 (4H, *m*), 2.00 (8H, *m*), 2.33 (1H, *m*), 2.26 (2H, *m*), 2.16 (1H, *m*), 1.41–1.20 (38H, *m*) and 0.89 (6H, *t*, *J* = 6.5)], a methoxyl (3H, δ_H 3.83, *s*) and three aromatic [δ_H 6.21 (1H, *s*), 6.14 (1H, *s*) and 5.97 (1H, *s*)] protons. The ^{13}CNMR spectrum of **2** (Table 2), which displayed two signals for carbonyl groups (δ_C 188.8, 182.4) and 10 resonances in the aromatic region (δ_C 101.3–159.3) supported the presence of 1,4-benzoquinone and phenyl rings. Furthermore, the spectrum also exhibited signals for a methoxyl (δ_C 56.4), olefinic (δ_C 130.1) and allylic (δ_C 27.4) carbons. Inspection on key HMBC correlations (Fig. 2) as well as comparison with literature data suggested the substitution pattern on the two rings, and a C4-C1′ linkage between the two rings (Fukuyama et al., 1993; Liu et al., 2009). Meanwhile, the HMBC correlations observed between H-7$_{ax}$ (δ_H 2.33) and C-2 (δ_C 182.2), C-3 (δ_C 147.3) and C-4 (δ_C 141.2), established the connectivity of one alkenyl chain at C-3, whereas correlations observed between H-7′ (δ_H 2.26) and C-1′ (δ_C 112.3), C-2′ (δ_C 143.1) and C-3′ (δ_C 108.6), placed the other alkenyl chain on C-2′. The length of the two alkenyl chains was as a C_{15} unit based on the ESI-MS (positive-ion mode) fragmentation pattern, where two fragment ion peaks corresponding to 1,4-benzoquinone (A) and phenyl (B) moieties at *m/z* 345 and 317, respectively, were observed.

As in the case of **1**, assignments of the double bonds in the alkenyl chains was accomplished by ozonolysis of the acetylated analogue (**2b**), which gave an aldehyde adduct (**2c**) having the sodiated molecular ion peak $[M+Na]^+$ at *m/z* 661.3328 in its HRESIMS (Scheme 2). On the basis of these considerations, the positions of the double bond were assigned to C-16, C-17 and C-16′, C-17′ carbons. The configurations of the double bonds on the alkenyl chains were deduced as *Z* based on chemical shift values of the allylic C-15 and C-18 carbons, which appeared as an overlapped

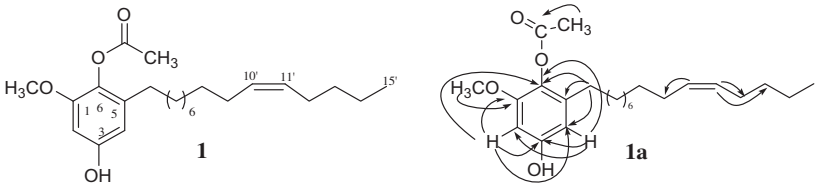
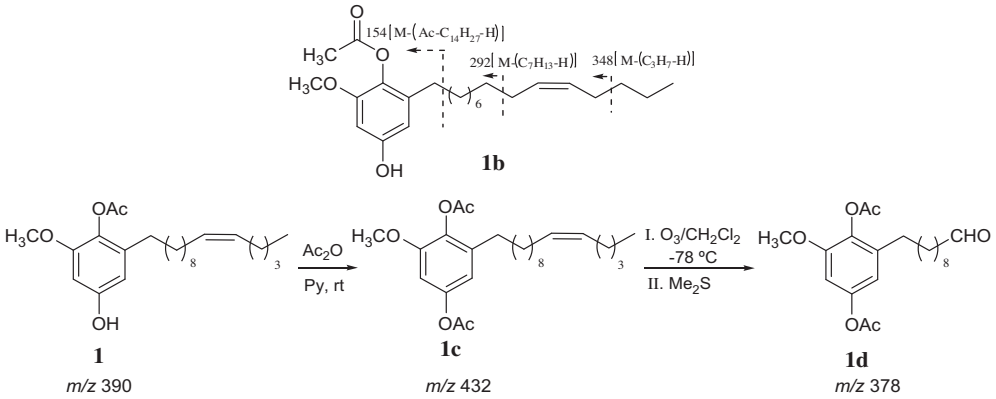


Fig. 1. Structure of compound **1** and its key HMBC correlations (1a).



Scheme 1. Key fragmentation (1B) and aldehyde adduct formation by ozonolysis of an acetylated analogue of **1**.

Table 2
^{1}H and ^{13}C NMR spectroscopic data of compounds **2** and **3** measured in $CDCl_3$.

Position	Compound **2**		Compound **3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	–	159.3	–	159.2
2	–	182.4	–	182.4
3	–	147.3	–	147.2
4	–	141.2	–	141.1
5	–	188.8	–	188.0
6	5.97 (*s*)	107.6	5.98 (*s*)	107.6
7eq	2.33 (*m*)	27.1	2.33 (*m*)	27.1
7ax	2.16 (*m*)	–	2.16 (*m*)	–
8–13	1.20–1.41(*m*)	28.4–30.1	1.15–1.41 (*m*)	28.4–30.3
14	1.41 (*m*)	28.4	1.41 (*m*)	28.4
15	2.00 (*m*)	27.4	2.01 (*m*)	27.4
16	5.32 (*m*)	130.1	5.35 (*m*)	130.1
17	5.32 (*m*)	130.1	5.35 (*m*)	130.1
18	2.00 (*m*)	27.4	2.01 (*m*)	27.4
19	1.20–1.41 (*m*)	32.2	1.26 (*m*)	32.2
20		22.6	1.26(*m*)	22.6
21	0.89 (*t*, *J* = 6.5 Hz)	14.3	0.88 (*t*, *J* = 6.5 Hz)	14.4
1-OCH_3	3.83 (*s*)	56.4	3.84 (*s*)	56.5
1′	–	112.3	–	112.6
2′	–	143.1	–	143.4
3′	6.21 (*s*)	108.6	6.28 (*s*)	108.6
4′	–	156.7	–	156.8
5′	6.14 (*s*)	101.3	6.16 (*s*)	101.2
6′	–	153.9	–	153.8
7′	2.26 (*m*)	33.8	2.23 (*m*)	33.7
8′–13′	1.20–1.41 (*m*)	28.4–30.1	1.15–141 (*m*)	28.4–30.3
14′	1.20 (*m*)	28.4	1.15–141 (*m*)	28.4–30.3
15′	2.00 (*m*)	27.4	1.15–141 (*m*)	28.4–30.3
16′	5.32 (*m*)	130.1	1.15–141 (*m*)	28.4–30.3
17′	5.32 (*m*)	130.1	1.15–141(*m*)	28.4–30.3
18′	2,00 (*m*)	27.4	1.15–141 (*m*)	28.4–30.3
19′	1.20 (*m*)	32.2	1.15–141 (*m*)	32.2
20′	1.41 (*m*)	22.6	1.15–141 (*m*)	22.6
21′	0.89 (*t*, *J* = 6.5 Hz)	14.3	0.88 (*t*, *J* = 6.5 Hz)	14.4

signal at δ_C 27.4 ppm (De Haan and Van de Ven, 1973; Liu et al., 2009).

The molecular formula of **3** was determined as $C_{43}H_{68}O_5$ by HRESI-MS, which gave an $[M+H]^+$at *m/z* 665.5136 (calcd. for $C_{43}H_{68}O_5$+H, 665.5140), and consistent with 10 degrees of unsaturation. The ^{1}H NMR spectrum of this compound was identical to **2**, except for the integration values of the olefinic and allylic protons, which were equivalent for only two and four protons, respectively (see Fig. 3). The position of the double bond on the alkenyl side-chain was confirmed at C-16′ and C-17′ based on ozonolysis of the acetylated analogue (**3b**) of **3**, which gave aldehyde adduct (**3c**) having the sodiated molecular ion peak $[M+Na]^+$ at *m/z* 717.4303 in its HRESIMS (Scheme 3). In a similar manner, as in the previous compounds, the configuration of the double bond was assigned as *Z* based on the chemical shift value of the allylic carbons (δ_C 27.4).

The attachment of the alkenyl moiety was established to be at the lower phenyl ring (ring-B) based on the results of ESI/MS/MS experiment conducted on compound **3**. Exertion of collision energy on the protonated molecular ion at *m/z* 665 led to the fragmentation, of which the prominent fragments at *m/z* 317 and 347 were assignable to the alkenylresorcinol **3d** and alkylbenzoquinone **3e** cations, respectively. On account of these arguments, **2** and **3** were therefore assigned labisiaquinone A and B, respectively. It is worth mentioning that labisiaquinone A has recently been reported (Ali and Khan, 2011) after our submission of this report for publication.

Sixteen other compounds were also isolated from this species including 1-*O*-methyl-6-acetoxy-5-pentadecylresorcinol (**4**) (Chang et al., 2009), 5-(pentadec-10*Z*-enyl)resorcinol (**5**) (Occolowitz and Wright, 1962), 5-(pentadecyl)resorcinol (**6**) (Suzuki et al., 1996), (−)-loliolide (**7**) (Kimura and Maki, 2001), stigmasterol (**8**) (Misra et al., 1984), hydroxyphenylethylamine (**9**) (Elgorashi et al., 2001), 3,4,5-trihydroxybenzoic acid (**10**) (Gottlieb et al., 1991), 3,4-dihydroxybenzoic acid (**11**) (Ban et al., 2007), (+)-catechin (**12**) (Nahrstedt et al., 1987), (−)-epicatechin (**13**) (Ban et al., 2006), kaempferol-3-*O*-α-rhamnopyranosyl-7-*O*-β-glycopyranoside (**14**) (Chen et al., 2007), kaempferol-4′-*O*-β-glycopyranoside (**15**) (Nørbæk and Kondo, 1999), quercetin-3-*O*-α-rhamnopyranoside (**16**) (Zhong et al., 1997), kaempferol-3-*O*-α-rhamnopyranoside (**17**) (Mizuno et al., 1990), (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**18**) (Liu et al., 2004) and stigmasterol 3-*O*-β-glycopyranoside (**19**) (Leitão et al., 1994). The identity of these compounds was confirmed

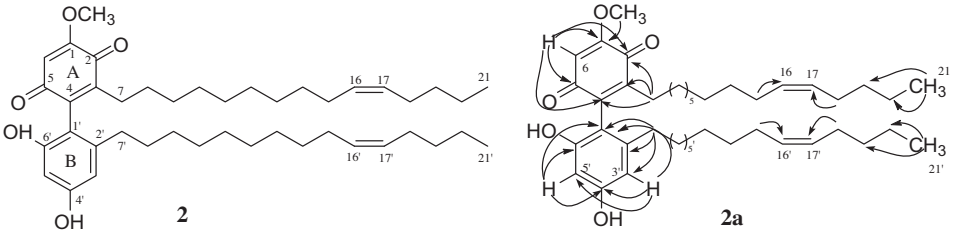
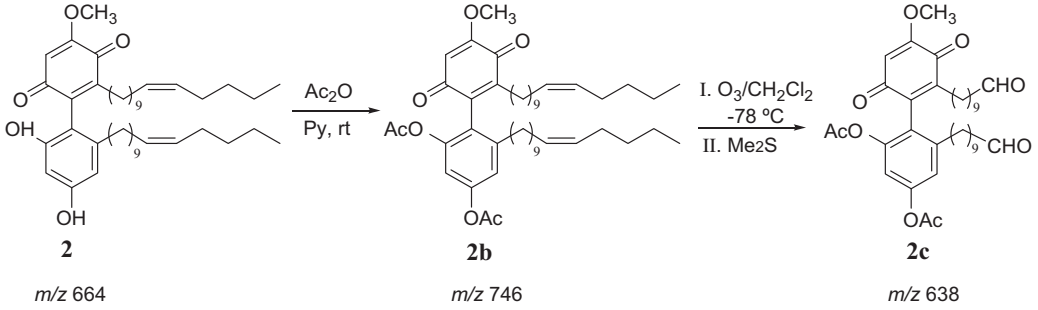


Fig. 2. Structure of compound **2** and its key HMBC correlations (2a).



Scheme 2. Aldehyde adduct formation by ozonolysis of an acetylated analogue of **2**.

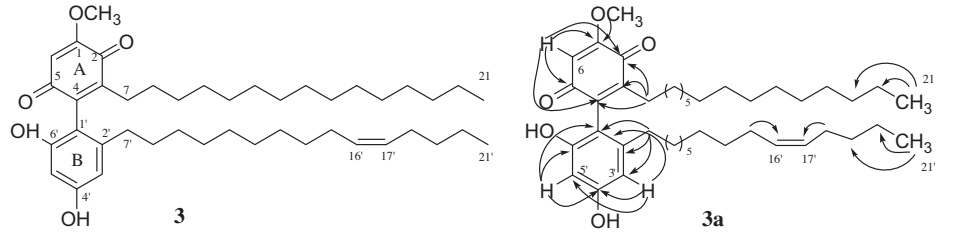
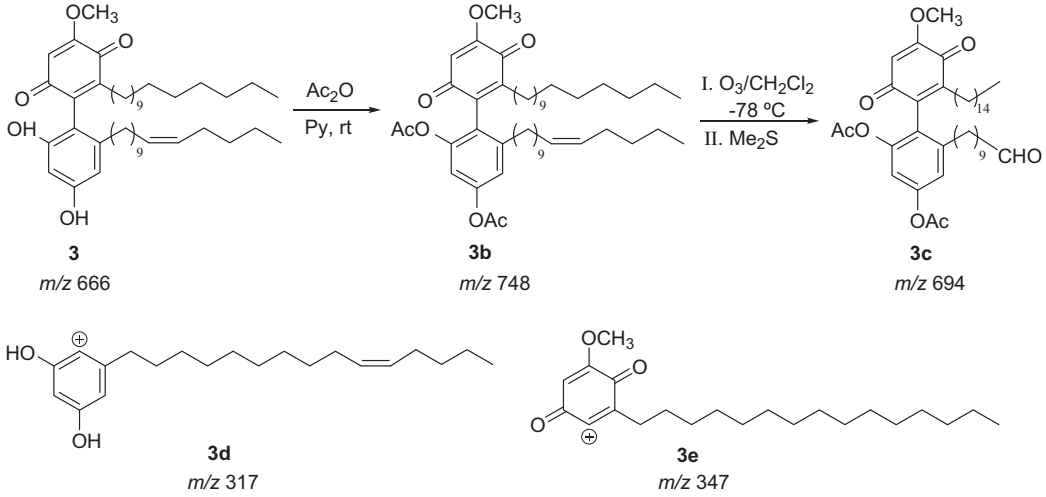


Fig. 3. Structure of compound **3** and its key HMBC correlations (3a).

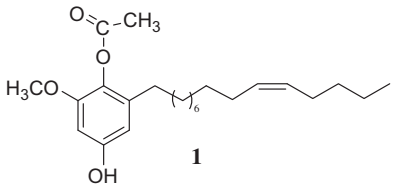
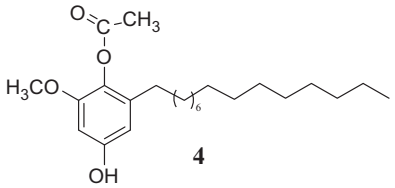


Scheme 3. Alkenyl resorcinol (3d), alkyl benzoquinone (3e) cation fragments, and aldehyde adduct formation by ozonolysis of an acetylated analogue of **3**.

by analysis of their spectroscopic data as well as by comparison of these and their physical properties, with those of the literature.

Twelve of the isolated compounds (**1**–**6**, **9**, **11**, **13**, **14**, **16** and **17**) were screened for cytotoxic activity at 10 μM against MCF-7 breast cancer cells. Only compounds displaying >50% cell death were selected for dose–response curves to obtain the three parameters (GI_{50}, TGI and LC_{50}). Compounds **1** and **4** displayed the desired activity in the preliminary screening and hence, the compounds were tested against PC-3, HCT-116 and MCF-7 cells to obtain the growth inhibitory parameters (Table 3). The benefit of testing against the three cell lines is it provides information on the potential selectivity of a compound. Although a limited number of cell lines were used for the assessment of cytotoxic selectivity, it gives an initial indication on the therapeutic value of a compound. It has

Table 3
In vitro cytotoxic activity of compounds **1** and **4** towards three cancer cell lines.

Compound	Growth inhibitory parameters (μM)			
	Cell line	GI_{50}	TGI	LC_{50}
 **1**	PC-3	0.3 ± 0.0	1.2 ± 0.0	8.0 ± 1.7
	HCT-116	0.3 ± 0.0	1.0 ± 0.1	7.7 ± 6.4
	MCF-7	0.4 ± 0.1	15.7 ± 2.1	41.3 ± 2.3
 **4**	PC-3	0.4 ± 0.1	1.6 ± 0.4	10.0 ± 4.4
	HCT-116	0.3 ± 0.0	1.6 ± 0.3	12.0 ± 8.7
	MCF-7	0.5 ± 0.1	15.0 ± 1.7	40.0 ± 0.0
Doxorubicin[a]	PC-3	0.7 ± 0.1	4.0 ± 0.2	20.0 ± 0.5
	HCT-116	0.6 ± 0.1	2.0 ± 0.2	5.0 ± 0.5
	MCF-7	0.6 ± 0.1	1.9 ± 0.2	5.5 ± 0.5

The cell lines used were: human prostate (PC-3), colon (HCT-116) and breast (MCF-7) cancer cells. The cells were treated for 96 h with at least four different concentrations of compounds ranging from 0.1 to 100 μM. MTT assay (Mosmann, 1983) was used to calculate GI_{50}, TGI and LC_{50} values (expressed in μM). Values are mean of three independent experiments and errors represent the SD values.

[a] Doxorubicin was used as a positive control.

been well established that compounds with cancer-type selectivity are beneficial in cancer chemotherapy. The GI_{50} values of **1** and **4** were similar in all the cell lines. However, when the TGI values were taken into consideration, the MCF-7 cells were less sensitive approximately by 15-fold when compared with PC-3 and HCT-116 cells. A similar pattern was observed at the LC_{50} level, in which the compounds were approximately 5-fold more active against PC-3 and HCT-116 cells as compared with MCF-7 cells. Therefore, although the selectivity was not discerned at the GI_{50} level, the compounds displayed selectivity for PC-3 and HCT-116 cells at the TGI and LC_{50} levels. The clinically used anticancer agent doxorubicin, which was used as a positive control, exhibited sub-micromolar to low micromolar growth inhibitory activities. It is of interest to note that **1** and **4** were more potent than doxorubicin at the GI_{50} level.

Structurally, **1** and **4** are closely related. While the alkyl chain in the former contains a double bond, the alkyl chain of the latter does not. The results therefore suggested that the occurrence of double bond moiety in the alkyl chain does not cause any difference in cytotoxic activity. Similar results have been observed on the relative phytotoxicity of lipid benzoquinones with various levels of unsaturation (Kagan et al., 2003). Cytotoxic activity of **4** and (8*Z*)-pentadecenyl isomer of **1** isolated from *Ardisia virens* and *A. brevicaulis*, respectively has been reported recently (Bao et al., 2010; Chang et al., 2009). These compounds also showed similar cytotoxic activity pattern based on their IC_{50} values towards A459, MCF-7, PANC-1, NCI-H460 and SF-268 cancer cell lines, although their growth inhibitory parameters (GI_{50}, TGI and LC_{50}) were not measured.

3. Concluding remarks

A comprehensive phytochemical investigation on the leaves of *L. pumila* led to the isolation of nineteen compounds (**1**–**19**), three of which (**1**–**3**) were new. Their structures were established on the basis of spectroscopic data, and the assignments of the double bond in the alkyl chain were confirmed by ozonolysis followed by ESI-MS analysis. In chemotaxonomic sense, the constituents of *L. pumila* are closely related to *Ardisia* species, a genus from the same Myrsinaceae family. Compounds **1** and **4** had the strongest cytotoxic activity at equipotent submicromolar growth inhibition (GI_{50}) in the tested cancer cell lines. More importantly, they exhibited cancer-type selectivity against PC-3 and HCT-116 cells at the TGI and LC_{50} levels.

4. Experimental

4.1. General experimental procedures

Melting points were recorded on a Koffler hot-stage apparatus and were uncorrected. The UV and IR spectra were recorded on a Varian UV–VIS 50 and Perkin–Elmer 1650 FTIR spectrophotometers, respectively. 1D NMR and 2D NMR spectra were acquired on a Varian Unity 500 MHz spectrometer either in $CDCl_3$, acetone-d_6, or CD_3OD. ESI–MS were determined using JEOL JMS-T 100LP spectrometer and HRESI–MS were determined using a FinniganLTQ-Orbitrap Discovery mass spectrometer. The chemical shifts (δ) were determined from residual solvent peaks. Column chromatography was performed using silica gel Merck 7734 (70–230 mesh ASTM), Merck 9385 (230–400 mesh ASTM)and reversed phase silica gel Merck 10167. Sephadex LH-20 (Fluka) was employed to purify the isolated compounds or remove chlorophyll. TLC analyses were carried out on Merck silica gel DC-plastikfolien 60 F_{254} plastic sheets with detection accomplished by spraying with 10% H_2SO_4 followed by heating at 100 °C, or by visualizing with a UV lamp at 254 and 366 nm. Semi-preparative HPLC system consisting of a JASCO pump PU-2089 plus and JASCO absorbance detector model UV-2077 plus 4-λ intelligent UV/VIS detector linked by JASCO ChromNAV version 1.09.03 software. Semi-preparative HPLC column used was XterraC18 OBD 5 μm (19× 150 mm), with the solvent system used was acetonitrile-H_2O (70:30–100:0, over 45 min) or (0:100–100:0) at flow rate 5 ml/min.

4.2. Plant material

Fresh leaves of *L. pumila* were obtained from Pahang which was supplier from F. A. Herbs Sdn. Bhd., Shah Alam, Malaysia in March

2008. They were cleaned, chopped into small pieces (3–5 mm thickness), and dried in the shade. A voucher specimen (ACP0084/08) was identified by Dr. Shamsul Khamis (A Resident Botanist) and deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia.

4.3. Extraction and isolation

The dried leaves (1.5 kg) were ground and extracted with MeOH–H_2O (80:20), three times at room temperature. The crude extract (about 214 g) was obtained after evaporating the solvent *in vacuo*. The crude extract was then fractionated by sequential liquid–liquid partitioning of H_2O and with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH to give 57.7, 5.4, 25.1 and 44.2 g of the respective fractions.

The *n*-hexane fraction (15 g) was subjected to flash column chromatography (CC) on reversed phase C18 column, eluted with MeOH–H_2O (8:2–1:0), to give 23 fractions. Fractions with similar TLC pattern were combined to yield five major combined fractions (H_1–H_5). Fraction H_2 (136 mg) was reapplied to a silica gel column eluted with *n*-hexane–EtOAc (1:0–0:1, v/v) to afford (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**18**, 19 mg). Further separation of fraction H_3 (3.5 g) by silica gel CC was performed with *n*-hexane–EtOAc (1:0–0:1, v/v) as eluent to afford four subfractions ($H_{3/1}$–$H_{3/4}$). Subfraction $H_{3/1}$ (1.515 g) was then subjected to semi-preparative reversed phase HPLC to yield four compounds, 5-(pentadec-10*Z*-enyl)resorcinol (**5**; 25.3 mg; t_R 21.5), 1-*O*-methyl-6-acetoxy-5-(pentadec-10*Z*-enyl)resorcinol (**1**; 45 mg; t_R 23.6), 5-(pentadecyl)resorcinol (**6**; 10 mg; t_R 25.5) and 1-*O*-methyl-6-acetoxy-5-pentadecylresorcinol (**4**; 39 mg; t_R 27.5). Fraction H_5 (0.525 mg) was purified by Sephadex LH-20 eluted with MeOH to afford three subfractions ($H_{5/1}$–$H_{5/3}$). Subfraction $H_{5/2}$ (395 mg) was then applied to semi-preparative reversed phase HPLC to yield labisiaquinone A (**2**; 120 mg; t_R 46.2) and labisiaquinone B (**3**; 42 mg; t_R 63.0). Subfraction $H_{5/3}$ (100 mg) was subjected to silica gel CC, eluted with *n*-hexane–EtOAc (4:1–0:1) to give stigmasterol-3-*O*-*β*-glycopyranoside (**19**, 22 mg).

The crude $CHCl_3$ fraction of *L. pumila* leaves (4.5 g) was fractionated by Sephadex LH-20 column, eluting with $CHCl_3$–MeOH (70:30 v/v) to give 22 subfractions, and all fractions with similar chromatograms were combined (based on TLC pattern) and evaporated to dryness under reduced pressure to afford four major fractions (C_1–C_4). Fraction C_1 (2.8 g) was separated by silica gel CC and eluted with $CHCl_3$–acetone (1:0–0:1) to give seven fractions ($C_{1/1}$–$C_{1/7}$). Fraction $C_{1/3}$ (47 mg) was further purified by silica gel CC, eluted with *n*-hexane–acetone (4:1–3:2) to give (−)-loliolide (**7**; 5 mg). Fraction C_3 (164 mg) was subjected to silica gel CC and eluted with $CHCl_3$–acetone (1:0–0:1), to give four subfractions ($C_{3/1}$–$C_{3/4}$). Fraction $C_{3/3}$ (36 mg) was further applied to a silica gel column to give kaempferol-3-*O*-α-rhamnopyranoside (**17**; 19 mg) after elution with $CHCl_3$–MeOH (4:1 v/v). Fraction C_4 (175 mg) was treated with MeOH to give stigmasterol (**8**; 5 mg) as a residue and the MeOH soluble was subjected to silica gel CC with $CHCl_3$–MeOH (4:1 v/v) as eluant to afford quercetin-3-*O*-α-rhamnopyranoside (**16**; 3 mg).

A portion of the EtOAc fraction (13 g) was subjected to reversed-phase silica gel CC, eluted with H_2O–MeOH (100:0–0:100, v/v) to yield 45 fractions. Each fraction (200 ml) was examined by TLC, and fractions with similar TLC patterns were combined to yield ten major fractions (E_1–E_{10}). Fraction E_1 (628 mg) was separated into four subfractions ($E_{1/1}$–$E_{1/4}$) by chromatography over Sephadex LH-20 with MeOH as eluent. The $E_{1/4}$ subfraction (198 mg) was subjected to silica gel CC eluted with $CHCl_3$–MeOH (100–0, 0–100, v/v) to give 4-hydroxyphenylethylamine (**9**; 15 mg). Fraction E_2 (600 mg) was fractionated using a solid-phase extraction (SPE) cartridge over normal-phase silica and eluted with $CHCl_3$–MeOH to give fractions $E_{2/1}$ (95 mg; from the 10% MeOH eluate), $E_{2/2}$ (312 mg; 35% MeOH) and $E_{2/3}$ (180 mg; 100% MeOH). Subfraction $E_{2/2}$ (312 mg) was fractionated by a Sephadex LH-20 column and eluted with MeOH to afford four fractions ($E_{2/2/1}$–$E_{2/2/4}$). Subfraction $E_{2/2/2}$ was subjected to semi-preparative RP HPLC to afford two pure compounds, 3,4,5-trihydroxybenzoic acid (**10**; 4 mg; t_R 17.9) and 3,4-dihydroxybenzoic acid (**11**; 3 mg; t_R 22.0). Fraction E_4 (1.31 g) was applied to normal-phase silica gel CC and eluting with a gradient polarity with MeOH (0–100%) in $CHCl_3$ to give three subfractions ($E_{4/1}$–$E_{4/3}$). The second subfraction $E_{4/2}$ (325 mg) was separated by Sephadex LH-20 column chromatography using MeOH as mobile phase to afford five subfractions ($E_{4/2/1}$–$E_{4/2/5}$), the subfraction$E_{4/2/5}$ (90 mg) was further separated by semi-preparative HPLC to give two pure compounds, (+)-catechin (**12**; 3 mg; t_R 24.75) and (−)-epicatechin (**13**; 8 mg; t_R 25.63). Fraction E_5 (1.45 g) was applied to a silica gel column using $CHCl_3$–MeOH (100–0, 0–100, v/v) for elution, which afforded six fractions ($E_{5/1}$–$E_{5/6}$). Fraction $E_{5/4}$ (235 mg) was passed through a Sephadex LH-20 column with MeOH as eluant to give three fractions ($E_{5/4/1}$–$E_{5/4/3}$). Subfraction $E_{5/4/2}$ (120 mg) was purified by semi-preparative HPLC to yield kaempferol-3-*O*-α-rhamnopyranosyl-7-*O*-β-glycopyranoside (**14**; 23 mg; t_R 25.81). Fraction E_7 (1.75 g) was separated over a Sephadex LH-20 column and eluted with MeOH to give four subfractions ($E_{7/1}$–$E_{7/4}$). Subfraction $E_{7/4}$ (531 mg) was subjected to silica gel CC ($CHCl_3$–MeOH gradient elution), yielding four fractions ($E_{7/4/1}$–$E_{7/4/4}$). Subfraction $E_{7/4/3}$ (276 mg) was further separated by semi-preparative HPLC to afford three pure compounds, kaempferol-4′-*O*-β-glycopyranoside (**15**; 3 mg; t_R 30.02), quercetin-3-*O*-α-rhamnopyranoside (**16**; 8 mg; t_R 30.35) and kaempferol-3-*O*-α-rhamnopyranoside (**17**; 120 mg, t_R 32.80).

4.4. Ozonolysis

Compound **1** (7.1 mg, 0.020 mmol) was dissolved in pyridine (0.5 ml) and Ac_2O (0.5 ml) and the resulting mixture was allowed to stand for 12 h at room temperature. The mixture was concentrated under reduced pressure to afford **1c** quantitatively. A stream of O_3 was bubbled into a solution of **1c** in CH_2Cl_2 (2.0 ml) for 0.5 min at -78 °C and excess ozone was removed by a stream of Ar. The reaction mixture was treated with Me_2S (0.1 ml) at −78 °C and the mixture was warmed to room temperature during the period of 3 h, and stirred for further 3 h at room temperature. The mixture was concentrated under reduced pressure and purified by PLC (EtOAc/*n*-hexane; 1:2) to afford **1d** (1 mg, 0.0026 mmol) as brown oil. The same reaction conditions of ozonolysis of **1** were applied for the ozonolysis of compounds **2** and **3** to give acetylated analogue (**2b**, **3b**) and an aldehyde adduct (**2c**, **3c**).

4.4.1. 1-O-Methyl-6-acetoxy-5-(pentadec-10Z-enyl)resorcinol (*1*)

Colorlessoil; UV (MeOH) λ_{max} nm (log ε): 229 (3.34), 274 (3.27); IR (thin film) ν_{max} cm^{-1}: 3437, 2926, 2857, 1747, 1609, 1191; negative ESI–MS: *m/z* 390 $[M-H]^-$, 347, 153, 292; positive HRESI–MS *m/z* 391.2852 $[M+H]^+$, (calcd. for $C_{24}H_{38}O_4$+H, 391.2843); for ^{1}HNMR (500 MHz, $CDCl_3$) and ^{13}CNMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 1.

4.4.2. Labisiaquinone A (*2*)

Red semisolid; UV (MeOH) λ_{max} nm (log ε): 268 (4.03), 223 (4.01); IR (KBr, disc) ν_{max} cm^{-1}: 3283, 2925, 2854, 2683, 1679, 1638, 1619, 1600, 1507, 1457, 1339, 1228, 1148, 1053, 847, 722; negative ESI–MS: *m/z* 661 $[M-H]^-$, 437, 345, 317; positive HR–ESI–MS *m/z* 663.4999 $[M+H]^+$, (calcd. for $C_{43}H_{66}O_5$+H, 663.4989); for ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}CNMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 2.

4.4.3. Labisiaquinone B (**3**)

Red semisolid; UV (MeOH) λ_{max} nm (logε): 271 (4.01), 220 (3.97); IR (KBr, disc) ν_{max} cm^{-1}: 3283, 2925, 2854, 2683, 1679, 1638, 1619, 1600, 1507, 1457, 1339, 1228, 1148, 1053, 847, 722; positive ESI–MS: *m/z* 665 $[M+H]^+$, 633, 582, 469, 439, 317; positive HR–ESI–MS *m/z* 665.5136 $[M+H]^+$, (calcd. for $C_{43}H_{68}O_5$+H, 665.5140); for ^{1}HNMR (500 MHz, $CDCl_3$) and ^{13}CNMR (125 MHz, $CDCl_3$) spectroscopic data, see Table 2.

4.5. Cytotoxic assay

4.5.1. Cell lines

Human cell lines representing cancers of the breast (MCF-7), colon (HCT-116) and prostate (PC-3) were obtained from the American Tissue Culture collection (Manassas, VA, USA).

4.5.2. Cell culture

All cell lines were maintained in RPMI-1640 (Life Technologies, Paisley, Scotland), supplement with 10% heat-inactivated fetal bovine serum FBS, (PAA Laboratories, Linz, Austria) 100 IU/mL penicillin and 100 μg/mL streptomycin (Life Technologies). Cells were grown in 25 cm^2 tissue culture flasks in a humidified atmosphere containing 5% CO_2 at 37 °C. Once the cells reach 80% confluence, 1ml of trypsin–EDTA solution was added to the flask for 5–10 min to detach the monolayer cells. The cells were occasionally observed under the inverted microscope until the cell layer was dispersed. Then, 3 ml of complete growth medium was added to the flask followed by repeated gentle pipetting to split apart the cell clumps. Approximately $0.5 - 1 \times 10^6$ cells were subcultured into a new 25 cm^2 flask containing 8 ml of fresh medium.

4.5.3. MTT colorimetric assay

The ability of **1** and **4** to induce growth inhibition on several cell lines was determined using MTT assay. The salt [3-(4,5-dimethylthiaszol-2-yl)-2,5-diphenyltetrazolium bromide] or MTT assay is commonly used in the screening of anti-cancer compounds, and this method was first developed in 1983 by "Mosmann (1983)". The tetrazolium salt (MTT) is used as a developing dye. The tetrazolium ring of MTT can be cleaved by dehydrogenases in the mitochondria of living cells to produce a purple formazan. The MTT soluble formazan reaction was only partially soluble in the medium, and so the DMSO was used to dissolve the formazan, and the optical densities at 550 nm are read by a scanning multi-well spectrophotometer (microplate reader) (VersaMax, Molecular Devices, Inc., USA).

Briefly, exponentially growing cells were seeded into 96-well plate at a density of approximately 2000 cells/0.18 ml/well and allowed to adhere overnight. Treatments ($n = 4$) in the final concentration range between 0.1 and 100.0 μM were introduced. Meanwhile, the control wells were treated with 0.01% of DMSO equivalent to the amount of DMSO used as a vehicle in the compound treated wells. After 96 h of incubation, 50 μl of MTT solution (2.0 mg/ml) was added and incubated for an additional 4 h. Medium and excessive MTT were aspirated and formazan formed was solubilized by the addition of 100 μl DMSO. Absorbance, as a measure of viable cell number, was read at 550 nm. Using the absorbance value on 0-day as optical density, the dose response growth curves were constructed and the following parameters: GI_{50} (the concentration of drugs that inhibits cell growth by 50%), TGI (the concentration of drugs that totally inhibits cell growth) and LC_{50} (the concentration of drugs that results in death to 50% of cell) were interpolated from the curves. The growth percentages were determined using the following formula: % of cell growth = $(A_T - A_0)/(A_C - A_0) \times 100$ *if* $A_T \geqslant A_0$, or % of cell growth = $(A_T - A_0)/A_C \times 100$ *if* $A_T < A_0$, A_T = absorbance of treated cells at day 4, A_C = absorbance of control cells at day 4, A_0 = absorbance on day 0.

Acknowledgements

The authors wish to thank Universiti Putra Malaysia for providing the financial under the Research University Grant Scheme (RUGS) No. 91178. The author (NHL) also wishes to thank the Scientific Chairs Unit, Taiban University for support.

References

Ali, Z., Khan, I.A., 2011. Alkyl phenols and saponins from the roots of *Labisia pumila* (Kacip Fatimah). Phytochemistry 72, 2075–2080.

Ban, J.Y., Jeon, S.-Y., Bae, K., Song, K.-S., Seong, Y.H., 2006. Catechin and epicatechin from *Smilacis chinae* rhizome protect cultured rat cortical neurons against amyloid [β] protein (25–35)-induced neurotoxicity through inhibition of cytosolic calcium elevation. Life Sci. 79, 2251–2259.

Ban, J.Y., Cho, S.O., Jeon, S.Y., Bae, K., Song, K.S., Seong, Y.H., 2007. 3,4-dihydroxybenzoic acid from *Smilacis chinae* rhizome protects amyloid [β] protein (25–35)-induced neurotoxicity in cultured rat cortical neurons. Neurosci. Lett. 420, 184–188.

Bao, L., Wang, M., Zhao, F., Zhao, Y., Liu, H., 2010. Two new resorcinol derivatives with strong cytotoxicity from the roots of *Ardisia brevicaulis* Diels. Chem. Biodiv. 7, 2901–2907.

Barr, J.R., Scannell, R.T., Yamaguchi, K., 1989. Structure elucidation of naturally occurring long-chain mono- and dienes. J. Org. Chem. 54, 494–496.

Chang, H.-S., Lin, Y.-J., Lee, S.-J., Yang, C.-W., Lin, W.-Y., Tsai, I.-L., Chen, I.-S., 2009. Cytotoxic alkyl benzoquinones and alkyl phenols from *Ardisia virens*. Phytochemistry 70, 2064–2071.

Chen, Y.-H., Chang, F.-R., Lin, Y.-J., Wang, L., Chen, J.-F., Wu, Y.-C., Wu, M.-J., 2007. Identification of phenolic antioxidants from Sword Brake fern (*Pteris ensiformis* Burm.). Food Chem. 105, 48–56.

Choi, H.-K., Kim, D.-H., Kim, J.W., Ngadiran, S., Sarmidi, M.R., Park, C.S., 2010. *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. J. Biosci. Bioeng. 109, 291–296.

Dat, N.T., Bae, K., Wamiru, A., McMahon, J.B., Le Grice, S.F.J., Bona, M., Beutler, J.A., Kim, Y.H., 2007. A dimeric lactone from *Ardisia japonica* with inhibitory activity for HIV-1 and HIV-2 ribonuclease H. J. Nat. Prod. 70, 839–841.

De Haan, J.W., Van de Ven, L.J.M., 1973. Configurations and conformations in acyclic, unsaturated hydrocarbons. A ^{13}C NMR study. Org. Mag. Resonance 5, 147–153.

Elgorashi, E.E., Drewes, S.E., Staden, J.v., 2001. Alkaloids from *Crinum moorei*. Phytochemistry 56, 637–640.

Fukuyama, Y., Kiriyama, Y., Okino, J., Kodama, M., 1993. Belamcandaquinones A and B, novel dimeric 1,4-benzoquinone derivatives possessing cyclooxygenase inhibitory activity. Tetrahedron Lett. 34, 7633–7636.

Gottlieb, H.E., Kumar, S., Sahai, M., Ray, A.B., 1991. Ethyl brevifolin carboxylate from *Flueggea microcarpa*. Phytochemistry 30, 2435–2438.

Horgen, F.D., Guinaudeau, H.L.N., Pezzuto, J.M., Soejarto, D.D., Farnsworth, N.R., Agcaoili, F., de los Reyes, G., Edrada, R.A., 1997. Isolation and structure elucidation of ardisenone: A new, cytotoxic alkenylphenol from *Ardisia iwahigensis*. J. Nat. Prod. 60, 533–535.

Ibrahim, J., Young-Hwa, K., Dae-Yeon, S., Byung, H.H., 1996. Inhibitory effects of malaysian medicinal plants on the platlet-activating factor (PAF) receptor binding. Nat. Prod. Sci. 2, 86–89.

Jaganath, N.L., 2000. Herbs. The green pharmacy of Malaysia. Kuala Lumpur: Vinpress Sdn. Bhd in collaboration with the Malaysian Agricultural Research and Development (MARDI).

Jamal, J.A., Houghton, P.J., Milligan, S.R., 1998. Testing of *Labisia pumila* for oestrogenic activity using a recombinant yeast screen. J. Pharm. Pharmacol. 50, 79–82.

Jamia, A.J., Houghton, P.J., 2000. Determination of iron content from *Labisia pumila* using inductively coupled plasma technique. In: Proceeding of 16th National Seminar on Natural Products, pp. 118–120.

Jimenez-Romero, C., Torres-Mendoza, D., Gonzalez, L.D.U., Ortega-Barria, E., McPhail, K.L., Gerwick, W.H., Cubilla-Rios, L., 2007. Hydroxyalkenylresorcinols from *Stylogyne turbacensis*. J. Nat. Prod. 70, 1249–1252.

Kagan, I.A., Rimando, A.M., Dayan, F.E., 2003. Chromatographic separation and in vitro activity of sorgoleone congeners from the roots of *Sorghum bicolor*. J. Agric. Food Chem. 51, 7589–7595.

Karimi, E., Jaafar, H.Z.E., Ahmad, S., 2011. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of *Labisa pumila* benth. Molecules 16, 4438–4450.

Kikuchi, H., Ohtsuki, T., Koyano, T., Kowithayakorn, T., Sakai, T., Ishibashi, M., 2009. Death receptor 5 targeting activity-guided isolation of isoflavones from *Millettia brandisiana* and *Ardisia colorata* and evaluation of ability to induce TRAIL-mediated apoptosis. Bioorg. Med. Chem. 17, 1181–1186.

Kimura, J., Maki, N., 2001. New loliolide derivatives from the Brown Alga Undaria pinnatifida. J. Nat. Prod. 65, 57–58.

Kobayashi, H., De Mejía, E., 2005. The genus *Ardisia*: A novel source of health-promoting compounds and phytopharmaceuticals. J. Ethnopharmacol. 96, 347–354.

Latiff, A., 1997. Medicinal and aromatic plants of Malaysia: Approaches to exploitation and conservation. In: Kamaruddin, M.S., Natesh, S., Asiah, O., Azizol, A.K. (Eds.), Proceedings of the Medicinal and Aromatic Plants of Malaysia: Strategies and Technologies for Conservation, 29–30 September 1997, Kuala Lumpur, Malaysia, pp. 20–31.

Leitão, S.G., Kaplan, M.A.C., Monache, F.D., 1994. Acylglucosylsterols from two *Aegiphila* species. Phytochemistry 36, 167–170.

Liu, J., Burdette, J.E., Sun, Y., Deng, S., Schlecht, S.M., Zheng, W., Nikolic, D., Mahady, G., van Breemen, R.B., Fong, H.H.S., Pezzuto, J.M., Bolton, J.L., Farnsworth, N.R., 2004. Isolation of linoleic acid as an estrogenic compound from the fruits of *Vitex agnus-castus* L. (chaste-berry). Phytomedicine 11, 18–23.

Liu, H., Zhao, F., Yang, R., Wang, M., Zheng, M., Zhao, Y., Zhang, X., Qiu, F., Wang, H., 2009. Dimeric 1,4-benzoquinone derivatives and a resorcinol derivative from *Ardisia gigantifolia*. Phytochemistry 70, 773–778.

Misra, T.N., Singh, R.S., Upadhyay, J., Srivastava, R., 1984. Isolation of a natural sterol and an aliphatic acid from *Vernonia cinerea*. Phytochemistry 23, 415–417.

Mizuno, M., Kyotani, Y., Iinuma, M., Tanaka, T., Iwatsuki, K., 1990. Kaempferol 3-rhamnoside-7-[6-feruloylglucosyl (1>3)rhamnoside] from *Asplenium prolongatum*. Phytochemistry 29, 2742–2743.

Mohamad, N., Mahmood, M., Mansor, H., 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. J. Med. Plants Res. 3, 217–223.

Mohamud, W., Nazaimoon, W., 2009. *Labisia pumila* extracts for reducing the risk of cardiovascular diseases, vol. WO 2009116848.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Nahrstedt, A., Proksch, P., Conn, E.E., 1987. Dhurrin, (−)-catechin, flavonol glycosides and flavones from *Chamaebatia foliolosa*. Phytochemistry 26, 1546–1547.

Nørbæk, R., Kondo, T., 1999. Flavonol glycosides from flowers of *Crocus speciosus* and *C. antalyensis*. Phytochemistry 51, 1113–1119.

Occolowitz, L., Wright, A., 1962. 5-(10-Pentadecenyl)resorcinol from *Grevillea pyramidalis*. Aust. J. Chem. 15, 858–861.

Ong, H.C., 2004. Tumbuhan Liar Khasiat Ubatan dan Kegunaan Lain.: Perpustakaan Negara Malaysia, Kuala Lumpur.

Rasadah, M.A., Nik-Musa'adah, M., Aznie-Aida, A., Mohd.-Rizal, A.K., 2001. Inhibitory activity of some selected malaysian medicinal plants on TPA induced mouse ear Oedema. In: Ahmad Sazali et al. (Eds.), Fine Chemicals from Natural Resources. University Publication Centre, University of Technology MARA, Shah Alam, Malaysia, pp. 371–376.

Singh, G.D., Ganjoo, M., Youssouf, M.S., Koul, A., Sharma, R., Singh, S., Sangwan, P.L., Koul, S., Ahamad, D.B., Johri, R.K., 2009. Sub-acute toxicity evaluation of an aqueous extract of *Labisia pumila*, a Malaysian herb. Food Chem. Toxicol. 47, 2661–2665.

Stone, B.C., 1988. Note on the genus *Labisia* Lindl. (Myrsinaceae). Malayan Nat. J. 42, 43–51.

Suzuki, Y., Esumi, Y., Hyakutake, H., Kono, Y., Sakurai, A., 1996. Isolation of 5-(8′Z-heptadecenyl)-resorcinol from etiolated rice seedlings as an antifungal agent. Phytochemistry 41, 1485–1489.

Tang, H.F., Lin, H.W., Chen, X.L., Cheng, G., Zhao, Y.P., Wen, A.D., 2009. Cytotoxic triterpenoid saponins from *Ardisia pusilla*. Chinese Chem. Lett. 20, 193–196.

Wiart, C., Wong, F.K.E., 2002. Medicinal Plants of Southeast Asia, 2nd ed. Prentice Hall, Petaling Jaya.

Zhong, X.-N., Otsuka, H., Ide, T., Hirata, E., Takushi, A., Takeda, Y., 1997. Three flavonol glycosides from leaves of *Myrsine seguinii*. Phytochemistry 46, 943–946.