J. Trop. Agric. and Fd. Sc. 45(2)(2017): 187 – 197

Phytochemicals and cytotoxic studies of *Zingiber cassumunar* Roxb.

(Fitokimia dan kajian sitotoksik *Zingiber cassumunar* Roxb.)

A. Mohd Zulkhairi[1], S. Mohd Aspollah[2], E. Gwendoline Cheng Lian[2] and A. Ahmad Bustamam[3]

[1]Agrobiodiversity and Environmental Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
[2]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
[3]UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience (IBS), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor

Absract

Zingiber cassumunar belongs to the family *Zingiberaceae* and is locally known as 'bonglai' in Malay and 'plai' among Thais. It has been traditionally used as treatment for women after giving birth or during confinement. Extraction and isolation of phytochemical compounds from the rhizome of *Zingiber cassumunar* Roxb. using various chromatographic techniques identified five compounds, namely, *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl]cyclo-hex-1-ene (1), (*E*)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol (2), 3,4-dimethoxybenzoic acid (3), 8-(13,14-dimethoxyphenyl)-2-methoxynaphto-1,4-quinone (4) and β-sitosterol (5). Structure elucidation of these compounds was carried out by various spectroscopic means. The cytotoxic activity of isolated compounds against human T-acute lymphoblastic leukemia cancer cells (CEMss) and human cervical cancer cells (HeLa) were carried out using MTT assay. Only the chloroform extract showed strong activity against CEMss cancer cells with IC_{50} values of 9.20 ± 0.02 µg/ml. Compounds (1), (2) and (4) showed strong cytotoxicity activity against HeLa cancer cells with IC_{50} values <15 µg/ml whilst compounds (1) and (4) demonstrated moderate cytotoxicity activity against CEMss cells with $1C_{50}$ values of 28.34 ± 0.39 µg/ml and 25.96 ± 0.94 µg/ml respectively. Therefore, *Zingiber cassumunar* has high potential to be commercially domesticated based on the anti-cancer properties of its compounds.

Keywords: Zingiberaceae, cytotoxicity, phenylbutenoid, ginger, *bonglai,* CEMss, HeLa

Introduction

The ginger family (*Zingiberaceae*) can be found in tropical climatic areas, mainly in South East Asian regions such as Thailand, Indonesia and Malaysia. It comprises about 1,200 species of which about 1,000 species are found in tropical Asia (Larsen 1999). Several genus belongs to this family, such as, *Kaempferia*, *Alpinia*, *Amomum*, *Curcuma*, *Costus* and *Zingiber* (Burkill 1935). Besides being used as one of the ingredients in spice, several other species are also traditionally consumed raw as 'Jamu', a concoction to enhance health (Habsah et al. 2000). The rhizomes from the genus of *Zingiber* normally grow on the ground

Article history
Received: 4.11.2015
Accepted: 5.10.2016

Authors full names: Mohd Zulkhairi Azid, Mohd Aspollah Sukari, Gwendoline Ee Cheng Lian and Ahmad Bustamam Abdul
E-mail: zulkhairi@mardi.gov.my

surface and bear leaf shoots close together (Kirtikar and Basu 1975). The commonly studied species, *Zingiber cassumunar*, locally known as 'bonglai' in Malaysia, 'bengle' in Java and 'plai' in Thailand, is a herbaceous plant that can grow up to approximately 1 – 2 meters high. It has aromatic leaves and its fleshy rhizomes are yellow in colour. It is locally used as treatment for woman after giving birth or during confinement (Burkill 1935).

Phenylbutenoid is a major secondary metabolite isolated from *Zingiber cassumunar* (Amatayakul et al. 1979; Kuroyanagi et al. 1980; Jitoe et al. 1993; Masuda and Jitoe 1995; Han et al. 2004; Nakamura et al. 2009; Sukari et al. 2009). *Zingiber cassumunar* shows various biological activities including anti-inflammatory (Ozaki et al. 1991; Jeenapongsa et al. 2003), antioxidant (Jitoe et al. 1994; Habsah et al. 2000), antibacterial (Habsah et al. 2000) and insecticidal (Nugroho et al. 1996). Meanwhile, the essential oils from different genus of Zingiberaceae collected from Pahang, Malaysia, including *Z. cassumunar*, were tested for its antimicrobial activity. The essential oils exhibited low or weak activity against several bacteria, namely, *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and two fungi, *Candida albicans* and *Cryptococcus neoformans* (Kamazeri et al. 2012). Apart from that, blended patches incorporating *Z. cassumunar* oils showed its potential as herbal medicine (Suksaeree et al. 2015). A remarkable finding from this species was its promising compound towards A549 (human lung carcinoma), SNU638 (human gastric cancer) and Col2 (human colon cancer) cells (Lee et al. 2007; Park et al. 2002). Theebaa et al. (2013) reported that *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3''', 4'''-dimethoxystyryl]cyclo-hex-1-ene (compound 1) had apoptogenic properties against human T-acute lymphoblastic leukemia (CEMss) cancer cells. Reported herein are the cytotoxic activities of compounds 1, 2 and 4 against human cervical (HeLa) cancer cells and compounds 2 and 4 against human T-acute lymphoblastic leukemia (CEMss) cancer cells.

Materials and methods

Plant materials

The rhizomes of *Z. cassumunar* were collected from Yogyakarta, Indonesia. The plant was identified by Professor Sugeng Riyanto. Voucher specimens (088/SR/07) were deposited in the Herbarium of Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

Extraction, isolation and purification

Approximately 700 g of finely ground rhizomes of *Z. cassumunar* was extracted using the cold maceration technique by first soaking the rhizomes in a non-polar solvent, petroleum ether, for 72 h at room temperature. Extractions were continued with chloroform, ethyl acetate and methanol. The extraction was repeated three times to remove the non-polar organic compounds, waxes and fats. The solvents were removed under reduced pressure and crude extracts were obtained. The separation from the crude extracts was carried out by vacuum liquid column chromatography (VLC), column chromatography (CC) with eluent of increase polarity of solvents. Various fractions collected were monitored by thin layer chromatography (TLC) profiling. Recrystallisation was carried out to obtain the homogenous and pure compounds prior to analysis after single spots were observed on the TLC plate.

Spectroscopic analysis

Isolated compounds were subjected to spectroscopic analysis. The IR spectra were recorded in a Perkin Elmer FTIR model 100 series spectrophotometer [applied samples directly-Universal Attenuated Total Reflection (UATR) Technique]. The molecular mass of the compounds were measured on a Shimadzu model

QP5050A with BPX5 for non-polar (5% phenylmethylsilane) capillary column (30 µm x 250 µm x 0.25 µm). Gas chromatography (GC) oven temperature was programmed from 50 – 265 °C at a rate 5 °C min^{-1} with an initial hold for 1 min and a final hold for 10 min, while MS operates at 70 eV. Meanwhile, the chemical structures of the compounds were elucidated by using JEOL Nuclear Magnetic Resonance (NMR) on 400 MHz and 500 MHz. Chemical shift (δ) was recorded in ppm relative to tetramethylsilane (TMS) signal (0.0 ppm) as an internal standard. The signals were described in terms of chemical shift with appropriate abbreviations of multiplicities. They were recorded as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet) and *m* (multiplet). The chemical shifts were recorded in ppm downfield from TMS.

Cytotoxicity assay

The cytotoxicity assay was carried out according to Mosmann (1983) with slight modifications. The human T-acute lymphoblastic leukemia cancer (CEMss) and human cervical cancer (HeLa) cells were procured from the American Type Culture Collection (ATCC), Maryland, USA. Trypsin EDTA, fetal calf serum, amphotericin B and penicillin-streptomycin were obtained from FlowLab (Australia). The microtetrazolium (MTT) powder was purchased from Amresco and the DMSO (dimethylsulphoxide) was purchased from Merck. Positive controls in this study, 5-fluorouracil and cisplatin were purchased from Sigma Aldrich. The medium was used to dilute the cells to a concentration of 5 x 10^3 cells/ml. From this cell suspension, 100 ml of various concentrations of the extracts were pipetted into a 96-well microtitre plate and incubated at 37 °C, 5% CO_2 for 72 h. The various concentrations used were 1,000, 500, 250, 125, 62.5 and 31.25 g/ml for crude extracts and 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml for compounds. The assays of each concentration of extracts were performed in triplicates and the control wells of untreated populations were also included. After 3 days, the fraction of surviving cells were determined relative to the untreated cell populations by the colorimeter MTT method where the viability of cells was measured by 20 µl of blue formazan crystal of MTT solution (5 mg/ml in PBS, freshly prepared before assay) added to each well at 37 °C for 4 h incubation. One hundred microliters of the medium was removed from each well. The plate was left at room temperature for 30 min before reading the absorbance. The absorbance was read with the Elisa reader test wavelength of 570 nm and a reference wavelength of 630 nm. The IC_{50} value was defined as the concentration of the test compound resulting in a 50% reduction of absorbance. Extracts and pure compounds which exhibited a cytotoxic index IC_{50} <10 µg/ml were considered to have significant cytotoxic activity (Mackeen et al. 1997). The assays were carried out in triplicate and the results were averaged.

Results and discussions

Extraction, isolation and purification

Five compounds labelled as 1, 2, 3, 4 and 5 were isolated from the crude extracts of *Z. cassumunar*. Isolation work from petroleum ether extract identified compounds 1, 2, 4 and 5. Extraction from chloroform extract also yielded compounds 1 and 4. Meanwhile, compound 3 was isolated from ethyl acetate extract.

Spectroscopic results

The chemical structures of all isolated compounds (*Figure 1*) were elucidated based on comparison of their physical and spectral data with reported literature values. Physical properties of compound 1 appeared as a white solid with a melting point range of 94 – 97 °C. The fourier transform infrared (FTIR) spectrum for compound 1, proved the cis configuration of H-1 and H-2 at cyclohexene ring from the presence of a strong absorption band at 1,649 cm^{-1} compared to trans configuration, that will

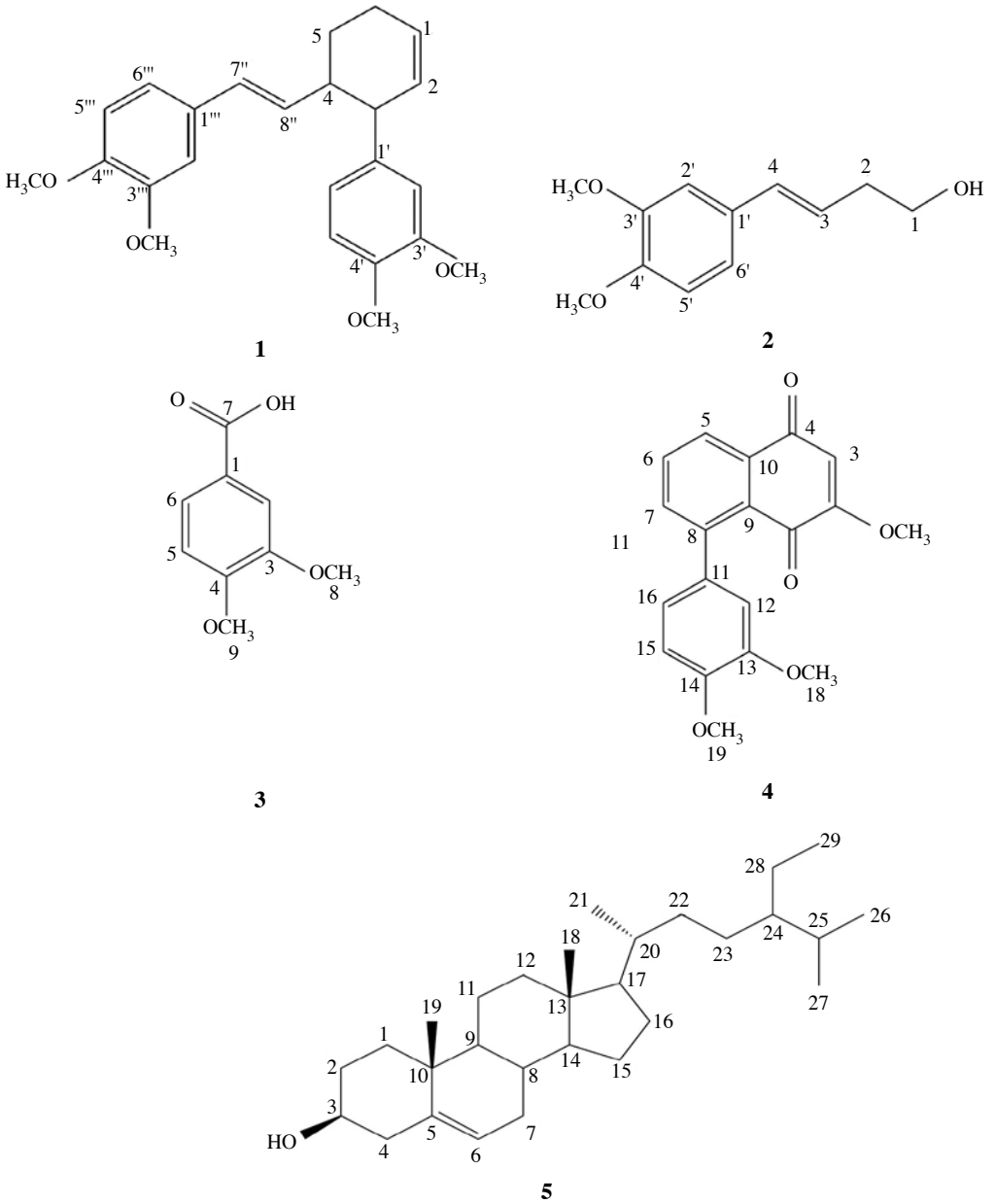


Figure 1. Structures of isolated compounds from ***Zingiber cassumunar***

give a vanishingly weak absorption band. Meanwhile, the molecular ion peak observed at m/z 380 corresponded to the molecular formula $C_{24}H_{28}O_4$. The ^1H NMR spectrum showed the presence of aromatic protons at δ 6.80 – 6.70 that integrated for six protons which were assigned to H-2', H-5', H-6', H-2''', H-5''' and H-6'''. Four singlets due to methoxy groups at 3.86 (3'-OCH3, 3H, s), 3.75 (4'-OCH3, 3H, s), 3.86 (3'''-OCH3, 3H, s) and 3.83 (4'''-OCH3, 3H, s) were observed. Twenty two peaks were observed with two peaks, each overlapping of two carbons at, δ 55.7 (3'-OCH3 and 4'-OCH3) and 55.8 (3'''-OCH3 and 4'''-OCH3) were observed from 13C NMR

spectrum. Consequently, compound 1 was identified as cis-3-(3',4'-dimethoxyphenyl)-4-[(E)-3''',4'''-dimethoxystyryl]cyclohex-1-ene in accordance with data reported by Kuroyanagi et al. (1980). Meanwhile, isomer of compound 1 which was identified as trans was previously isolated by Han et al. (2004).

Isolation and purification from petroleum ether extract yielded compound 2 which appeared as a pale yellow oil**.** The FTIR spectrum showed a broad absorption band at 3,391 cm^{-1} which indicated the presence of a hydroxyl group. The *trans* H-4 and H-3 protons were observed at δ 6.41 (1H, *d*, *J* = 16.5 Hz) and 6.06 (1H, *dt, J* = 16.5, 6.4 Hz) respectively, to give *E* configuration of compound 2. The molecular ion peak of compound 2 was observed at *m/z* 208 corresponding to the molecular formula $C_{12}H_{16}O_3$. The ^1H NMR spectrum of compound 2 showed a doublet at δ 6.90 (1H, *d*, *J* = 1.8 Hz) identified for H-2'. Another doublet at 6.78 (1H, *d*, *J* = 8.3 Hz) and doublet of doublet at 6.88 (1H, *dd*, *J* = 8.3, 1.8 Hz) were due to H-5' and H-6' respectively. The ^{13}C NMR spectrum showed presence of twelve peaks with signals at δ 149.1 and 148.6 which attributed to C-3' and C-4' respectively, while methoxyl carbons of 3'-OCH_3 and 4'-OCH_3 were assigned at δ 56.0 and 55.9. Compound 2 was further identified as (*E)*-4-(3',4'-dimethoxyphenyl)but-3-en-1-ol in accordance with previous data (Masuda and Jitoe 1995).

Compound 3 appeared as a white solid with a melting point range of 180 – 182 °C. From the IR spectrum, the presence of a hydroxyl group was confirmed by a broad absorption band at 3,442 cm^{-1} and C-O bond at 1,272 cm^{-1}. The molecular ion was observed at *m/z* 182 corresponding to the molecular formula $C_9H_{10}O_4$ while base peak was at *m/z* 168. In the ^1H NMR spectrum, the signals for aromatic protons were in good agreement with ABX splitting pattern whereby H-6 proton at δ 7.79 (1H, *dd*, *J* = 8.3, 1.9 Hz) ortho-coupled with H-5 proton at 6.93 (1H, *d*, *J* = 8.3 Hz), besides meta-coupled to H-2 (δ 7.60, 1H, *d*, *J* = 1.9 Hz). Based on above-mentioned spectral data comparison with previous report (Machida and Kikuchi 1996), compound 3 was identified as 3,4-dimethoxybenzoic acid or also known as veratric acid.

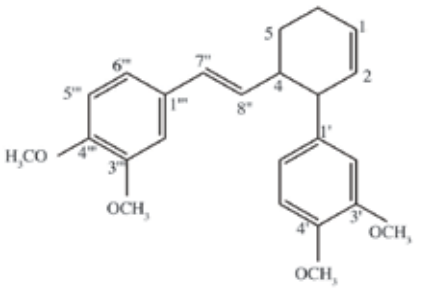
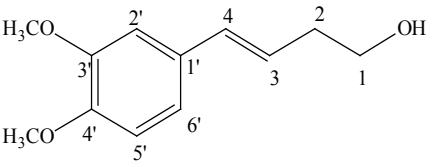
An orange needle crystal 4 was obtained from the isolation work from petroleum ether extract with a melting point range of 179 – 181 °C. The molecular ion peak of compound 4 was observed at 324 which corresponded to the molecular formula of $C_{19}H_{16}O_5$. The presence of carbonyl groups was then proved at the absorption band 1,617 cm^{-1} from the FTIR spectrum. The ^1H NMR spectrum of compound 4 showed a total of sixteen protons including signals at δ 8.14 (H-5), 7.69 (H-6), 7.53 (H-7), 6.90 (H-15), 6.78 (H-16), 6.76 (H-12) and 6.14 (H-3). Two obvious peaks were observed in ^{13}C NMR spectrum at δ 184.9 and 180.0 owing to carbonyl groups which were then identified as C-4 and C-1. According to previous data (Amatayakul et al. 1979), compound 4 was identified as 8-(13,14-dimethoxyphenyl)-2-methoxynaphto-1,4-quinone).

Compound 5 was isolated from chloroform extract as a white solid. The melting point of compound 5 was at a range of 130 – 132 °C. The presence of a double bond (C=C) group was indicated with an absorption band of 1,636 cm-1, while absorption at 1,056 cm-1 corresponded to the (C-O) stretching band. Apart from that, absorption bands at 2,936 and 2,868 cm^{-1} were due to C-H bond. The molecular ion peak observed at m/z 414 corresponded to the molecular formula of $C_{29}H_{50}O$. The ^1H and ^{13}C NMR data of β-sitosterol were compared with previous literature from Kojima et al. (1990). Details of full assignments of spectroscopic data of all isolated compounds (1 – 5) are tabulated in *Table 1*.

Cytotoxicity assay

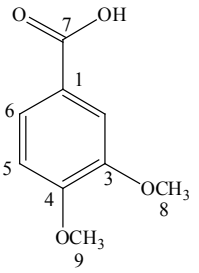
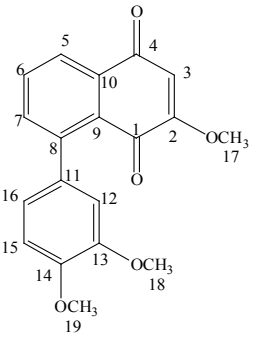
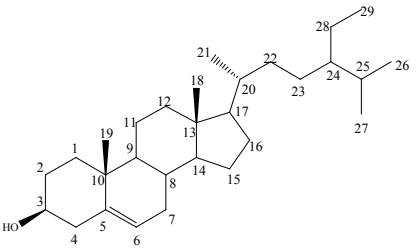
Cytotoxicity activity of crude extracts and selected compounds were carried out against

Table 1. Full assignment of spectroscopic data of compounds 1 – 5 isolated from rhizomes of *Zingiber cassumunar*

Comp.	Chemical structure/Physical properties	Spectral data
1	 *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl]cyclohex-1-ene White solid, m.p. 94 – 97 °C (Lit. m.p., 99 – 100 °C) (Kuroyanagi et al. 1980)	**IR υ_{max}** (cm^{-1}, UATR): 3018 (=C-H), 2929 (C-O), 1649, 1588 (C=C), 1509, 1458, 1233, 1138, 1019, 853, 785, 680, 614. **Mass spectrometry (MS) *m/z* (% intensity)**: 380 (M^+, 15), 300 (2), 229 (2), 190 (100), 175 (17), 159 (80), 144 (17). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 6.80 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.76 (3H, *br.s*, H-6',H-6''',H-5'''), 6.73 (1H, *s*, H-2'''), 6.70 (1H, *s*, H-2'), 6.26 (1H, *d*, *J* = 16.0 Hz, H-7''), 5.81 (1H, *dt*, *J* = 10.1, 3.6 Hz, H-1), 5.99 (1H, *d*, *J* =10.1 Hz, H-2), 5.59 (1H, *dd*, *J* =16.0, 9.0 Hz, H-8''), 3.86 (3H, *s*), 3.75 (3H, *s*), 3.86 (3H, *s*), 3.83 (3H, *s*), 3.51 (1H, *br. s*, H-3), 2.72 (1H, *m*, H-4), 2.22 (2H, *m*, H-6), 1.68 (2H, *t*, *J* = 7.0 Hz, H-5). **^{13}C NMR (100 MHz, $CDCl_3$)**: δ 148.9 (C-3'''-OCH_3), 148.2 (C-4''' -OCH_3), 148.0 (C-3'-OCH_3), 147.5 (C-4'-OCH_3), 133.8 (C-1'), 132.4 (C-8''), 131.0 (C-1'''), 128.0 (C-2), 128.5 (C-7''), 129.0 (C-1), 121.9 (C-6'), 118.7 (C-6'''), 113.7 (C-2'), 111.1 (C-5'''), 110.3 (C-5'), 108.9 (C-2'''), 55.7 (C-3'''-OCH_3 and 4'''-OCH_3), 55.8(C-3'''-OCH_3 and 4'''-OCH_3) 45.8 (C-3), 42.6 (C-4), 24.8 (C-6), 24.3 (C-5) (Kuroyanagi et al. 1980)
2	 (*E*)-4-(3',4'-dimethoxyphenyl)but-3-en-1-ol Pale yellow oil	**IR υ_{max}** (cm^{-1}, UATR): 3391 (OH), 2935 (=C-H), 2837 (C-H), 1591 (C=C), 1511, 1257, 965, 800, 761. **MS *m/z* (% intensity)**: 208 (M^+, 65), 177 (100), 162 (12), 146 (77), 131 (35), 91 (38). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 6.90 (1H, *d*, *J* = 1.8 Hz, H-2'), 6.88 (1H, *dd*, *J* = 8.3 , 1.8 Hz, H-6'), 6.78 (1H, *d*, *J* = 8.3 Hz, H-5'), 6.41 (1H, *d*, *J* = 16.5 Hz, H-4), 6.06 (1H, *dt*, *J* = 6.4, 16.5 Hz, H-3), 3.88 (3H, *s*, OCH_3), 3.85 (3H, *s*, OCH_3), 3.72 (2H, *t*, *J* = 6.4 Hz, H-1), 2.44 (2H, *q*, *J* = 6.4 Hz, H-2). **^{13}C NMR (100 MHz, $CDCl_3$)**: δ 149.1 (C-3'), 148.6 (C-4'), 132.5 (C-4), 130.5 (C-1'), 124.5 (C-3), 119.2 (C-6'), 111.2 (C-5'), 108.6 (C-2'), 62.2 (C-1), 56.0 (C-3'-OCH_3), 55.9 (C-4'-OCH_3), 36.4 (C-2) (Masuda and Jitoe 1995)

(*cont.*)

Table 1. (Cont.)

Comp.	Chemical structure/Physical properties	Spectral data
3	 3,4-dimethoxybenzoic acid White solid, m.p. 180 – 182 °C (Lit. m.p. 181.5 – 182 °C) (Kuroyanagi et al. 1980)	**IR υ_{max}** (cm^{-1}, UATR): 3442 (OH), 3004, 2940, 1680 (C=O), 1468, 1272, 762. **MS *m/z* (% intensity)**: 182 (M^+, 10) 168 (100), 154 (5), 153 (83), 125 (24), 108 (5), 97 (45), 79 (15), 63 (15), 51 (45), 45 (15). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 7.60 (1H, *d, J* = 1.9 Hz, H-2), 6.93 (1H, *d*, *J* = 8.5 Hz, H-5), 7.79 (1H, *dd,* *J* = 8.5, 1.9 Hz, H-6), 3.95 (3H, *s*, 8-OCH_3), 3.96 (3H, *s*, 9-OCH_3).**^{13}C NMR (100 MHz, $CDCl_3$)**: δ 171.7 (C-7), 153.6 (C-4), 148.6 (C-3), 124.6 (C-6), 121.7 (C-1), 112.2 (C-5), 110.2 (C-2), 56.0 (C-8), 56.0 (C-9) (Kuroyanagi et al. 1980)
4	 8-(13,14-dimethoxyphenyl)-2-methoxynaphto-1, 4-quinone Orange needle crystal, m.p. 179-181ºC (Lit m.p. 181 – 182 °C) (Amatayakul et al. 1979)	**IR υ_{max}** (cm^{-1}, UATR): 2940 (=C-H), 1617 (C=O), 1460 (C=C), 1221, 1026, 754, 383. **MS *m/z* (% intensity)**: 324 (M^+, 48), 309 (20), 293 (100), 266 (13), 263 (5), 250 (15), 223 (15), 195 (15), 181 (5), 152 (15), 139 (25). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 8.14 (1H, *d*, *J* = 8.1 Hz, H-5), 7.69 (1H, *t*, *J* = 6.9 Hz, H-6), 7.53 (1H, *d*, *J* = 8.1 Hz, H-7), 6.90 (1H, *d*, *J* = 6.4 Hz, H-15), 6.78 (1H, *dd*, *J* = 9.2, 1.8 Hz, H-16), 6.76 (1H, *d*, *J* = 1.8 Hz, H-12), 6.13 (1H, *s*, H-3), 3.90 (3H, *s,* 14-OCH_3), 3.84 (3H, *s,* 13-OCH_3), 3.83 (3H, *s,* 2-OCH_3).**^{13}C NMR (100 MHz, $CDCl_3$)**: δ 184.9 (C-4), 180.0 (C-1), 161.1 (C-2), 148.7 (C-13), 148.6 (C-14), 144.0 (C-10), 137.3 (C-7), 133.6 (C-8,C-11), 133.2 (C-6), 128.3 (C-9), 126.1 (C-5), 120.5 (C-16), 111.8 (C-12), 111.0 (C-15), 108.5 (C-3), 56.5 (C-2-OCH_3), 56.0 (C-13-OCH_3, 14-OCH_3) (Amatayakul et al. 1979)
5	 β-sitosterol White solid, m.p. 130 – 132 °C (Lit. m.p. 136 – 138 °C) (Zhang et al. 2005)	**IR υ_{max}** (cm^{-1}, UATR): 3442 (OH), 2936, 2868, 1636 (C=C), 1462, 1378, 1056. **MS *m/z* (% intensity)**: 414 (M^+, 48), 396 (20), 255 (25), 159 (28), 105 (35), 81 (48), 55 (100). **^{1}H NMR (400 MHz, $CDCl_3$)**: δ 5.36 (IH, *d*, *J* = 8.0 Hz, H-6), 3.52 (1H, *m,* H-3), 1.01 (3H, *s*, H-19), 0.93 (*d*, *J* = 6.5 Hz, H-21), 0.85 (*d*, *J* = 8.0 Hz, H-29), 0.83 (*d*, *J* = 6.5 Hz, H-26), 0.81 (*d*, *J* = 6.5 Hz, H-27), 0.68 (*s*, H-18).**^{13}C NMR (100 MHz, $CDCl_3$)**: δ 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-13), 42.3 (C-4), 39.8 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-8), 31.9 (C-7), 29.1 (C-25), 31.6 (C-2), 28.2 (C-16), 26.0 (C-23), 24.3 (C-15), 23.0 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 11.9 (C-18) (Zhang et al. 2005)

CEMss and HeLa cancer cells. Results were expressed as IC_{50} (µg/ ml) ± standard deviation from three repeated experiments and the assay was carried out in triplicate. All crude extracts of *Z. cassumunar* showed no cytotoxicity against CEMss cells with IC_{50} values >30 µg/ml except for the chloroform extract, which gave IC_{50} of 9.20 ± 0.02 µg/ml. Compounds 1 and 4 demonstrated moderate cytotoxicity against CEMss cells with IC_{50} values of 28.34 ± 0.39 µg/ml and 25.96 ± 0.94 µg/ml respectively. Meanwhile, all crude extracts showed negative results against HeLa cancer cells with IC_{50} values >30 µg/ml. Surprisingly, compounds 1, 2 and 4 which were isolated from petroleum ether and chloroform extracts showed strong cytotoxicity against HeLa cancer cells with IC_{50} values <15 µg/ml. Compounds 1, 2 and 4 showed synergistic effect which individually exhibited moderate and strong cytotoxicity against HeLa cancer cells compared to their origin extract.

In 1999, Vimala et al. reported that several species from the Zingiberaceae family, such as *Curcuma domestica*, *Kaempferia galanga* and *Z. Cassumunar* contained naturally occurring non-toxic compounds that inhibit the 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) in Raji cells. The findings suggest that the potential extract of several species from the Zingiberaceae family including *Z. cassumunar* could be further studied as it can contribute to anti-tumour promoting activity. Previously, isomers of compound 1 isolated in 2004 showed a promising cytotoxic activity towards A549, SNU638 and Col2 cancer cells (Han et al. 2004). Previously, *in vitro* cytotoxicity effect of compound 1 on various cancer cells and also normal human blood mononuclear cells and the involvement of its apoptogenic properties were reported. It was found to be selectively active against CEMss cancer cells but did not show any cytotoxic activity towards normal human blood mononuclear cells (IC_{50} >50 µg/ml). Further assessments of its morphology revealed that compound 1 demonstrated distinctive morphological changes corresponding to typical apoptosis (Theebaa et al. 2013). The findings concluded that compound 1 demonstrated apoptogenic properties against CEMss cancer cells which led to programmed cell death via intrinsic mitocondrial pathway of apoptosic induction.

Phenolic compounds are known to have antioxidant capacity to scavenge free radicals which leads to oxidative stress. They are believed to suppress the transformative, hyper proliferative and inflammatory processes that initiate carcinogenesis (Shukla and Singh 2007). Phenolic compounds are also suggested to possess potent antioxidant and anticancer activities (Cai et al. 2004). Hence, the phenolic group consisting of compounds 1, 2 and 4 may suggest the potency against CEMss and HeLa cancer cells.

The cytotoxic activities of the crude extracts and selected compounds are summarised in *Table 2*. This is the first

Table 2. Cytotoxicity activities of crude extracts and isolated compounds from rhizomes of *Zingiber cassumunar*

Extracts/ compounds	Cytotoxic activities (IC50 µg/ml)	
	CEMss	HeLa
Petroleum ether	>30	>30
Chloroform	9.20 ± 0.02	>30
Ethyl acetate	>30	>30
Methanol	>30	>30
1	28.34 ± 0.39	14.61 ± 0.29
2	>30	14.30 ± 0.29
3	>30	>30
4	25.96 ± 0.94	10.49 ± 1.09
5	NT	NT
5-fluorouracil	1.54 ± 0.035	NT
Cisplatin	NT	0.05

Note: CEMss (Human T-acute lymphoblastic leukemia cells); HeLa (Human cervical cancer cells). IC_{50} >30: not active; NT: Not tested. *Results are expressed as IC_{50} values (µg/ml) ± standard deviation of three experiments performed in triplicate

report on the preliminary cytotoxic activities of isolated phenylbutenoid against human cervical cancer cells (HeLa) (compounds 1, 2 and 4) and human T-acute lymphoblastic leukemic cancer cells (CEMss) (compounds 2 and 4).

Conclusion

Extraction from rhizomes of *Z. cassumunar* identified five compounds. Chloroform extract showed strong cytotoxic activity against CEMss cancer cells with IC_{50} <10 ug/ml. Meanwhile compounds 1, 2 and 4 showed compelling interesting cytotoxic activity against CEMss and HeLa cancer cells. Isolated compounds 1, 2 and 4 showed promising cytotoxic activity against cancer cells and can be further developed as potential anti cancer drugs.

Acknowledgements

Authors would like to acknowledge Ministry of Science, Technology and Innovation (MOSTI), Malaysia, for awarding the National Science Fellowship (NSF). Highest gratitude is extended to Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, Serdang, for the facilities provided for the isolation and elucidation of isolated compounds and UPM-MAKNA Cancer Research Laboratory at the Institute of Bioscience, Serdang, Malaysia, for the facilities and the assistance provided for the cytotoxicity assay.

References

Amatayakul, T., Cannon, J.R., Dampawan, P., Dechatiwangsa, T., Giles, R.G.F., Huntrakul, C., Kusamran, K., Mokkhasamit, M., Raston, C.L., Reutrakul, V. and White, A.H. (1979). Chemistry and crystal structure of some constituents of *Zingiber cassumunar*. *Australian Journal of Chemistry* 32: 71 – 88

Theebaa, A., Ahmad Bustamam, A., Mohd Aspollah, S., Siddig Ibrahim, A., Syam, M., Behnam, K., Mohd Zulkhairi, A., Nabilah, M.N., Reenaa Joys, A., Ng, K.B., Hamid, A.H. and Heshu, S.R. (2013). A phenylbutenoid dimer, *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl] cyclohex-1-ene, exhibits apoptogenic properties in T-acute lymphoblastic leukemia cells via induction of p53-independent mitochondrial signaling pathway. *Evidence-Based Complimentary and Alternative Medicine,* Volume 2013, Article ID 939810, 14 pages. Hindawi Publishing Corporation. Retrieved from http://dx.doi.org/10.1155/2013/939810

Burkill, I.H. (1935). A dictionary of the economic products of the Malay Peninsula, Vol (2): 2335 – 2336, London: Government of the Straits Settlements & Federated Malay Stat by the Crown agents for the colonies

Cai, Y., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Science* 74: 2157 – 2184

Habsah, M., Amran, M., Mackeen, M.M., Lajis, N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A. and Ali, A.M. (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmacology* 72: 403 – 410

Han, A.R., Min, H.Y., Windono, T., Jeohn, G.H., Jang, D.S., Lee, S.K. and Seo, E.K. (2004). A new cytotoxic phenylbutenoid dimer from the rhizomes of *Zingiber cassumunar. Planta Medica* 70: 1095 – 1097

Jeenapongsa, R., Yoovathaworn, K., Sriwatanakul, K.M., Pongprayoon, U. and Sriwatanakul, K. (2003). Anti-inflammatory activity of (*E*)-1-(3, 4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb. *Journal of Ethnopharmacology* 87: 143 – 148

Jitoe, A., Masuda, T. and Mabry, T.J. (1994). Novel antioxidants, cassumunarin A, B and C, from *Zingiber cassumunar. Tetrahedron Letters* 35(7): 981 – 984

Jitoe, A., Masuda, T. and Nakatani, N. (1993). Phenylbutenoid dimers from the rhizomes of *Zingiber cassumunar*. *Phytochemistry* 32(2): 357 – 363

Kamazeri, T.S.A.T., Samah, O.A., Taher, M., Susanti, D. and Qaralleh, H. (2012). Antimicrobial activity and essential oils of *Curcuma aeruginosa, Curcuma mangga* and *Zingiber cassumunar* from Malaysia. *Asian Pacific Journal of Tropical Medicine* 5(3): 202 – 209

Kirtikar, K.R. and Basu, B.D. (1975). Indian medicinal plants, (Basu, B.D., ed.). Delhi: Periodical Express: 4 – 6

Kojima, H., Sato, N., Hatano, A. and Ogura, H. (1990). Sterol glucosides from *Prunella vulgaris*. *Phytochemistry* 29(7): 2351 – 2355

Kuroyanagi, M., Fukushima, S., Yoshihira, K., Natori, S., Dechatiwongse, T., Mihashi, K., Nishi, M. and Hara, S. (1980). Further characterization of the constituents of a Thai medicinal plant, *Zingiber cassumunar* Roxb. *Chemical Pharmaceutical Bulletin* 28(10): 2948 – 2959

Larsen, K. (1999). Gingers of Peninsular Malaysia and Singapore (Wong, K.M., ed.). Kota Kinabalu, Sabah: Natural History Publications (Borneo): 5 – 10

Lee, J.W., Min, H.Y., Han, A.H., Cheng, H.J., Park, E.J., Park, H.J., Hong, J.Y., Seig, E.K. and Lee, S.K. (2007). Growth inhibition and induction of G1phase cell cycle arrest in human lung cancer cells by a phenylbutenoid dimer isolated from *Zingiber cassumunar. Biological and Pharmaceutical Bulletin* 30(8): 1561

Machida, K. and Kikuchi, M. (1996). Norisoprenoids from Viburnum Dilatatum. Phytochemistry 41 (5): 1333 – 1336

Mackeen, M.M., Ali, A.M., El-Sharkawy, S.H., Manap, M.Y., Salleh, K.M., Lajis, N.H. and Kawazu, K. (1997). Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. *International Journal of Pharmacognosy* 35: 174 – 178

Masuda, T. and Jitoe, A. (1995). Phenylbutenoid monomers from the rhizomes of *Zingiber cassumunar*. *Phytochemistry* 39(2): 459 – 461

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. *Journal of Immunological Method* 65: 55 – 63

Nakamura, S., Iwami, J., Matsuda, H., Wakayama, H., Pongpiriyadcha, Y. and Yoshikawa, M. (2009). Structures of new phenylbutenoids and nitric acids production inhibitors from the rhizomes of *Zingiber cassumunar*. *Chemical and Pharmaceutical Bulletin* 59(11): 1267 – 1272

Nugroho, B.W., Schwarz, B., Wray, V. and Proksch, P. (1996). Insecticidal constituents from rhizomes of *Zingiber cassumunar* and *Kaempferia rotunda*. *Phytochemistry* 41(1): 129 – 132

Ozaki, Y., Kawahara, N. and Harada, M. (1991). Anti-inflammatory effect of *Zingiber cassumunar* Roxb. and its active principles. *Chemical Pharmaceutical Bulletin* 39(9): 2353 – 2356

Park, G., Lee, E.J., Min, H.Y., Choi, H.Y., Han, A.R., Lee, S.K. and Seo, E.K. (2002). Evaluation of cytotoxic potential of Indonesian medicinal plants in cultured human cancer cells. *Natural Product Sciences* 8(4): 165 – 169

Shukhla, Y. and Singh, M. (2007). Cancer preventive properties of ginger: A brief review. *Food & Chemical Toxicology* 45: 683 – 690

Sukari, M.A., Azid, M.Z., Soh, P.A., Ho, M.S., Ee, G.C.L., Abdul, A.B. and Ali, N.A. (2009). Chemical constituents from rhizomes of *Zingiber cassumunar. Ultra Science* 21(3): 711 – 715

Suksaeree, J., Charoenchai, L., Madaka, F., Monton, C., Sakunpak, A., Charoonratana, T. and Pichayakorn, W. (2015). *Zingiber cassumunar* blended patches for skin application: formulation, physico-chemical properties, and *in vitro* studies. *Asian Journal of Pharmaceutical Sciences* 10(4): 341 – 349

Vimala, S., Norhanum, A.W. and Yadav, M. (1999) Anti-tumour promoter activity in Malaysian medicinal ginger rhizobia used in traditional medicine. *British Journal of Cancer* 80(1/2): 110 – 116

Zhang, X., Geofrray, P., Miesch, M., Julien-David, D., Raul, F., Aoude-Werner, D. and Marchioni, E. (2005). Gram-scale chromatographic purification of β-sitosterol synthesis and characterization of β-sitosterol oxides. *Steroids* 70: 886 – 895

Abstrak

Zingiber cassumunar Roxb. tergolong daripada famili Zingiberaceae dan ia dikenali sebagai bonglai dan plai oleh masyarakat Melayu dan Thai masing-masing. Secara tradisionalnya, ia digunakan untuk rawatan kaum wanita selepas bersalin atau ketika berpantang. Pengekstrakan dan pengasingan daripada rizom *Z. cassumunar* Roxb. dengan pelbagai teknik kromatografi seperti kromatografi kolum telah memberikan lima sebatian yang telah dikenal pasti sebagai cis-3-(3',4'-dimetoksifenil)-4-[(*E*)-3''',4'''-dimetoksistiril]siklo-hek-1-ena (1), (*E*)-4-(3',4'-dimetoksifenil)but-3-en-1-ol (2), 3,4-dimetoksibenzoik asid (3), 8-(13,14-dimetoksifenil)-2-metoksinafto-1,4-kuinon (4) dan beta-sitosterol (5). Elusidasi struktur untuk kesemua sebatian tersebut dilakukan menggunakan pelbagai kaedah spektroskopi. Aktiviti sitotoksik sebatian terpencil terhadap sel kanser T-akut leukemia limfoblastik manusia (CEMss) dan sel kanser serviks manusia (HeLa) telah dijalankan menggunakan esei antiproliferasi MTT. Hanya ekstrak klorofom menunjukkan kesan aktiviti yang kuat terhadap sel kanser CEMss dengan nilai IC_{50} 9.20 ± 0.02 μg/ml. Sebatian 1, 2 dan 4 menunjukkan kesan sitotoksik aktiviti yang kuat terhadap sel kanser HeLa dengan nilai IC_{50} <15 μg/ml manakala sebatian 1 dan 4 menunjukkan kesan sitotoksik yang sederhana terhadap sel kanser CEMss dengan IC_{50} 28.34 ± 0.39 μg/ml dan 25.96 ± 0.94 μg/ml masing-masing. Oleh itu, *Z. cassumunar* dianggap mempunyai potensi yang tinggi untuk dikomersialkan berdasarkan kebaikan sebatian terpencilnya terhadap sel kanser manusia.