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Cytotoxic and cytostatic effects of four Annonaceae plants on human cancer cell lines

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Abstract

Several species of the Annonaceae plants have been used as complementary medicine for cancer-associated illnesses in some ethnic groups of northern Thailand. This study investigated the cytotoxic and cytostatic activity of methanolic extracts derived from the stems of these plants, including *Uvaria longipes* (Craib) L.L.Zhou, Y.C.F.Su & R.M.K. Saunders, *Artabotrys burmanicus* A.DC, *Marsypopetalum modestum* (Pierre) B.Xue & R.M.K.Saunders, and *Dasymaschalon* sp. Cell death induction of seven human cancer cell lines and cell cycle analyses were assessed by Annexin V and/or propidium iodide (PI) staining and analyzed by flow cytometry. Treatment of cancer cell lines with the extract of four Annonaceae plants resulted in various cytotoxic activities depending on cell type. The extract of *U. longipes* exhibited the highest cytotoxic activity capable of inducing cell death of several cancer cell lines, particularly against hepatocellular carcinoma cell lines (HepG2 and Hep3B). This extract was capable of inducing cell cycle arrest at the SubG1 phase. Phytochemical screening of all the extracts revealed the presence of alkaloids, sterols, tannins, anthraquinone glycoside, coumarin, and flavonoids. Determination of active compounds by high-performance liquid chromatography standards revealed bullatacin and asiminecin in all the extracts. The extract of Annonaceae stem or its compounds may provide an opportunity for the development of new therapies against cancer.

Keywords Annonaceae · Anti-cancer activity · Cell cycle arrest · Apoptosis · Bullatacin · Asiminecin

Introduction

Ethnomedicinal plants are sources of active compounds that have been used in many countries as food and as traditional medicine to treat a wide range of diseases (Amoo *et al.* 2014; Tariq *et al.* 2015, 2016; Dinda *et al.* 2016; Maroyi 2017; Odonne *et al.* 2017). The World Health Organization reported that interest in traditional medicine continues to grow around the world (WHO 2013). Ethnomedicinal plants possess various pharmacological and biological properties, including antioxidant, anti-inflammatory, anti-microbial, anti-plasmodial, and anti-helminthic activities (Amoo *et al.* 2014; Tariq *et al.* 2015, 2016; Dinda *et al.* 2016; Maroyi 2017; Odonne *et al.* 2017). Several ethnomedicinal plants contain cytotoxic compounds capable of inducing apoptotic cell death and have been used as anti-cancer agents (Jung *et al.* 2018a, b; Mirza *et al.* 2018; Yang *et al.* 2018; Ye *et al.* 2018).

Induction of apoptotic cell death is one of the promising approaches to targeting cancer cells. This process involves two main pathways that are extrinsic and intrinsic (Elmore 2007). The extrinsic pathway is stimulated when the death receptors are engaged with specific signaling molecules, while the intrinsic pathway is directly promoted by cellular sensing of extracellular and/or intracellular stresses. Both apoptotic pathways require suitable stimuli to initiate the process (Elmore 2007). Exploration of active compounds from natural products

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capable of activating the apoptotic process holds potential for alternative and/or complementary treatment of cancer.

The Annonaceae family of plants consist of 112 genera and approximately 2500 species which most of them are found in the tropics (Quilez *et al.* 2018). Several species of the Annonaceae family are rich in secondary metabolites, such as alkaloids, terpenoids, flavonoids, and acetogenins (Moreira *et al.* 2013). A number of alkaloid derivatives, such as jerantinine B (Qazzaz *et al.* 2016), liriodenine (Li *et al.* 2017), and vinorelbine (Capasso 2012), are apoptotic agents capable of inhibiting cell proliferation. Commonly known as custard apple, *Annona squamosa* Linn contains terpenoid capable of inducing apoptosis of hepatoma cells via a mitochondria-mediated pathway (Chen *et al.* 2017). Extracts from *Annona muricata* containing flavonoids inhibit the growth of the human promyelocytic leukemia (HL-60) cells, probably through the disruption of MMP, reactive oxygen species (ROS) generation, and the G0/G1 cell arrest (Pieme *et al.* 2014). In addition, bullatacin, an acetogenin isolated from the fruit of *Annona atemoya*, can induce apoptosis of human hepatocarcinoma cell line (2.2.15 cells) in a time- and dose-dependent manner (Chih *et al.* 2001). These data imply the potential use of the *Annonaceae* plants as alternative and/or complementary medicine for cancer treatment.

We have previously shown that the extracts from leaves of four species of the Annonaceae plant, namely *Uvaria longipes* (Craib) L.L.Zhou, Y.C.F.Su, & R.M.K.Saunders; *Dasymaschalon* sp.; *Artabotrys burmanicus* A.D.C.; and *Marsypopetalum modestum* (Pierre) B.Xue & R.M.K. Saunders induced cell cycle arrest and apoptosis on human cancer cell lines (Pumiputavon *et al.* 2017). In general, different parts of plants contain different chemical constituents. The present study was conducted to evaluate the cytotoxic activity of stem-derived methanolic extracts from these plants. The extracts were tested against human cervical carcinoma, human hepatocellular carcinoma, and human hematopoietic cell lines *in vitro*. High-performance liquid chromatography (HPLC) was also performed to identify the bioactive components in all the extracts.

Materials and Methods

Cell lines and culture Human cervical carcinoma (HeLa and SiHa) (a kind gift from Assoc. Prof. Tipaya Ekalaksananan, Khon Kaen University, Thailand), human hepatocellular carcinoma (HepG2 and Hep3B) (a kind gift from Prof. Duncan R. Smith, Mahidol University, Thailand), and human myeloid leukemia (K562, U937, and RAJI) (a kind gift from Prof. Sumalee Tungpradabkul, Mahidol University, Thailand) cells were cultured in the Roswell Park Memorial Institute (RPMI) (Gibco-BRL, Grand Island, NY) medium containing 10 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Merck Millipore, Darmstadt, Germany), 1 mM of sodium bicarbonate (RCI LABSCAN, Bangkok Thailand), 10% fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml) (Gibco-BRL) (RPMI complete media) at 37°C in a 5% CO_2 incubator. All cell lines have been authenticated by DNA Forensics Laboratory Pvt. Ltd., India.

Plant materials and extraction *Uvaria longipes* (collection no.: Chaowasku 132), *Dasymaschalon* sp. (collection no.: Chaowasku 120), and *Marsypopetalum modestum* (collection no.: Chaowasku 164) were collected from private residences at coordinates 13.790384, 100.372378; and *Artabotrys burmanicus* (collection no.: Chaowasku 163) was collected from a private garden at coordinates 13.919300, 99.952555 as described previously (Pumiputavon *et al.* 2017). The stems of the plant materials were washed with distilled water to remove dust, air dried, and ground into powder. Dry powders were soaked in methanol in large containers at room temperature for 24 h. The solvent extract was collected and filtered through filter paper. The filtrate was concentrated under

Table 1 IC50 values of stem-derived extracts in cancer cell lines

Crude	IC_{50} value (μg/ml)						
	HeLa	SiHa	HepG2	Hep3B	K562	U937	RAJI
SUL[a]	131	653.5	126.9	119.6	879.4	1676.1	1522.8
SD[b]	658.2	1008.6	89.1	661.9	1019.4	955.7	2373.5
SAB[c]	2490.2	1780.4	474.2	1162.1	10,403.8	3984.1	1678.1
SMM[d]	10,782	731.2	990.1	2415.5	14,833.3	1678.1	2800

[a] Extract obtained from the stem of *U. longipes*

[b] Extract obtained from the stem of *Dasymaschalon* sp.

[c] Extract obtained from the stem of *A. burmanicus*

[d] Extract obtained from the stem of *M. modestum*

Table 2 Average (± SD) percentages of dead cells identified from dot plots of Annexin V and PI, with statistical analysis

	Concentration of extracts (μg/ml)				Control	
	1000	500	250	125	UT-C[a]	DMSO-C[b]
HeLa						
SUL[c]	89.5 ± 4.1**	94.4 ± 2.4**	61.1 ± 2.3**	54.0 ± 1.7**	3.1 ± 1.5	3.2 ± 1.3
SD[d]	65.3 ± 1.2**	53.8 ± 4.6**	27.2 ± 1.1**	14.1 ± 6.5**		
SAB[e]	20.6 ± 3.1**	10.3 ± 2.1**	3.5 ± 0.7	4.6 ± 0.9		
MM[f]	10.3 ± 2.3**	12.4 ± 4.5**	8.8 ± 6.5**	5.1 ± 1.1		
SiHa						
SUL[c]	62.5 ± 4.4**	42.3 ± 3.8**	24.2 ± 1.7**	5.9 ± 0.8	5.2 ± 1.7	8.6 ± 1.4
SD[d]	51.7 ± 4.5**	23.1 ± 0.2**	11.4 ± 4.2*	14.6 ± 0.5**		
SAB[e]	45.1 ± 3.2**	5.3 ± 1.5	4.0 ± 1.4*	2.0 ± 0.0**		
SMM[f]	61.1 ± 3.2**	40.3 ± 1.5**	38.0 ± 1.4**	12.0 ± 0.0**		
HepG2						
SUL[c]	97.2 ± 3.3**	93.6 ± 1.0**	74.8 ± 2.7**	49.0 ± 1.6**	11.1 ± 6.6	12.4 ± 6.6
SD[d]	96.1 ± 7.9**	92.1 ± 1.2**	84.6 ± 1.4**	68.9 ± 1.8**		
SAB[e]	89.0 ± 0.7**	39.4 ± 0.4**	36.8 ± 0.4**	35.5 ± 1.2**		
SMM[f]	49.7 ± 8.5**	54.8 ± 1.8**	47.2 ± 2.5**	34.1 ± 0.1**		
Hep3B						
SUL[c]	92.2 ± 1.3**	92.8 ± 1.9**	79.2 ± 2.4**	50.0 ± 0.4**	8.1 ± 0.6	6.9 ± 0.9
SD[d]	69.6 ± 4.3**	2.6 ± 0.3**	24.1 ± 2.8**	10.4 ± 0.4**		
SAB[e]	47.0 ± 2.4**	18.6 ± 4.0**	19.3 ± 1.1**	13.3 ± 1.1**		
SMM[f]	24.7 ± 0.4**	15.5 ± 3.1**	10.6 ± 3.0**	9.2 ± 0.5**		
K562						
SUL[c]	59.1 ± 1.4**	25.2 ± 0.8**	18.4 ± 0.9**	11.7 ± 0.1**	5.6 ± 1.3	5.0 ± 0.1
SD[d]	52.5 ± 0.6**	18.7 ± 1.6**	14.2 ± 1.3**	10.2 ± 1.4**		
SAB[e]	9.6 ± 0.3**	6.2 ± 0.2*	5.0 ± 0.7	5.4 ± 0.2		
SMM[f]	8.6 ± 1.3**	7.5 ± 1.0*	5.7 ± 0.3	6.3 ± 1.7		
U937						
SUL[c]	32.3 ± 0.9**	8.3 ± 0.3**	5.8 ± 0.7	5.4 ± 0.3	7.4 ± 1.4	5.7 ± 0.7
SD[d]	57.1 ± 0.5**	3.8 ± 1.3**	5.0 ± 0.5	5.9 ± 0.1		
SAB[e]	16.5 ± 0.8**	5.0 ± 0.3	5.3 ± 0.3	5.2 ± 0.1		
SMM[f]	31.3 ± 5.9**	5.0 ± 3.7	4.5 ± 0.1*	4.1 ± 0.2*		
RAJI						
SUL[c]	34.3 ± 0.9**	8.3 ± 0.3**	5.8 ± 0.7	5.4 ± 0.3	5.6 ± 2.2	7.0 ± 3.1
SD[d]	30.6 ± 7.2**	26.6 ± 1.1**	19.3 ± 0.6**	18.2 ± 2.7**		
SAB[e]	77.2 ± 2.6**	29.3 ± 0.3**	29.2 ± 0.4**	29.2 ± 0.9**		
SMM[f]	34.0 ± 1.1**	26.4 ± 1.1**	35.2 ± 2.6**	20.8 ± 1.0**		

The means of percentages of apoptotic cell death followed by an asterisk (*) indicate statistically significant difference from the DMSO-treated control at $P < 0.05$. "" means statistically significant difference from DMSO-treated control at $P < 0.01$.**

[a] Untreated control

[b] DMSO-treated control

[c] Extract obtained from the stem of *U. longipes*

[d] Extract obtained from the stem of *Dasymaschalon* sp.

[e] Extract obtained from the stem of *A. burmanicus*

[f] Extract obtained from the stem of *M. modestum*

vacuum in a rotary evaporator at 40°C. All the methanolic extracts were resuspended in dimethyl sulfoxide (DMSO) (Gibco-BRL) at a concentration of 100 mg/ml.

Annexin V staining assay Cell death of human cancer cell lines (HeLa, SiHa, HepG2, Hep3B, K562, U937, and RAJI) was analyzed based on the Annexin V/propidium iodide (PI) dual

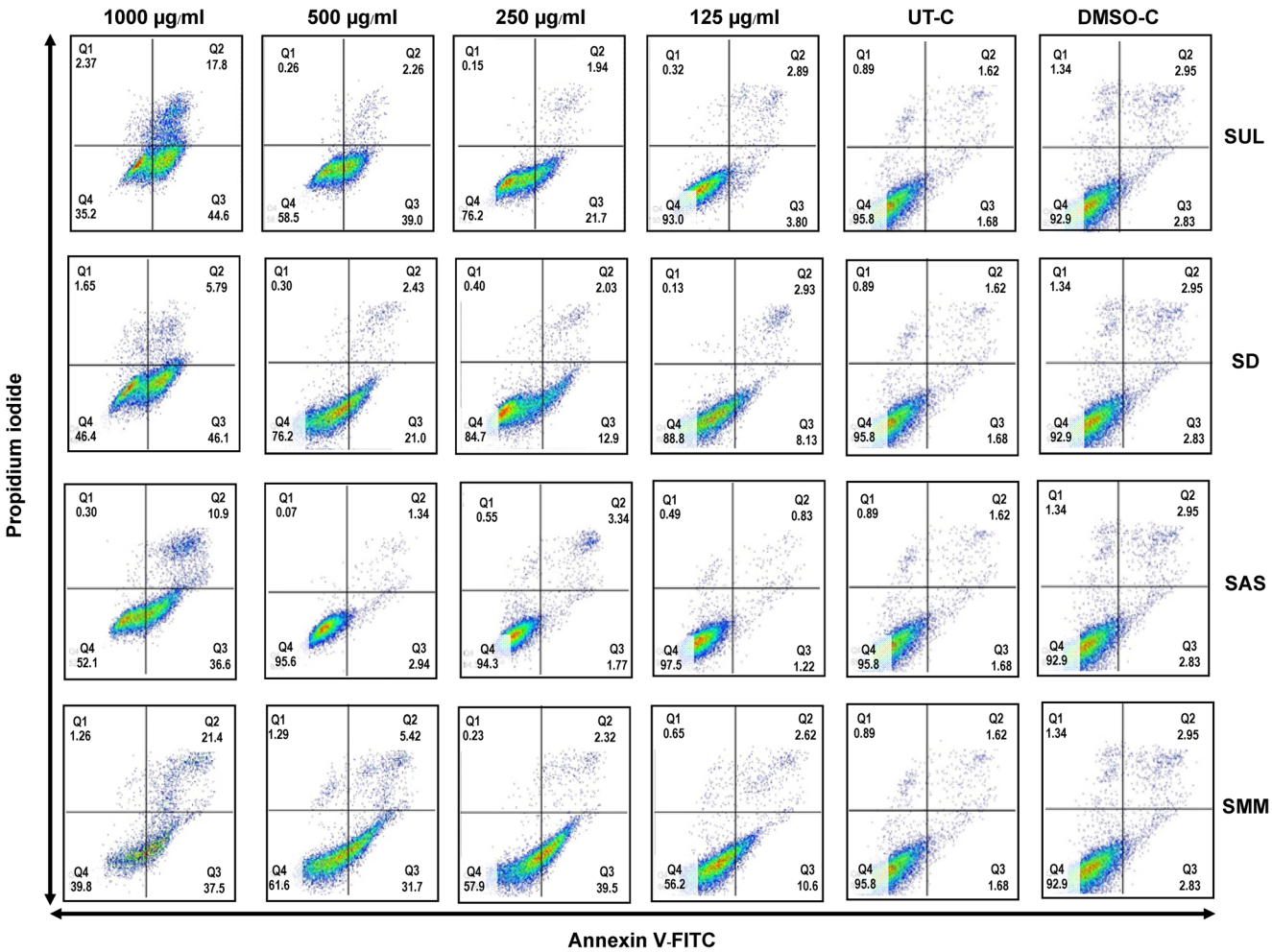

Figure 1. Effect of extracts obtained from the stems of *U. longipes* (*A*), *Dasymaschalon* sp. (*B*), *A. burmanicus* (*C*), and *M. modestum* (*D*) on the induction of apoptosis in the sample SiHa cell. SiHa cells were cultured in the presence of various concentrations of the crude extracts for 24 h. The cells were then stained with Annexin V and PI, and analyzed by flow cytometry.

Table 3 Average (± SD) percentages of cells in sub-G1 phase obtained from histogram of PI staining, with statistical analysis

	Concentration of extracts (µg/ml)				Control	
	1000	500	250	125	UT-C[a]	DMSO-C[b]
HeLa	43.2 ± 1.1**	34.2 ± 0.8**	13.4 ± 0.9**	8.0 ± 3.9*	4.0 ± 1.2	6.2 ± 2.7
SiHa	55.6 ± 0.5**	34.3 ± 3.4**	27.5 ± 2.0**	23.2 ± 2.1**	5.6 ± 1.3	6.6 ± 0.5
HepG2	67.8 ± 1.66**	27.6 ± 0.4**	20.3 ± 1.9**	27.9 ± 0.4**	6.2 ± 1.6	2.8 ± 0.7
Hep3B	4.3 ± 0.17	5.3 ± 0.2	5.7 ± 0.5	6.7 ± 0.5	4.2 ± 4.2	5.4 ± 0.8
K562	55.6 ± 0.5**	38.3 ± 0.4**	27.5 ± 2.7**	23.2 ± 3.1**	5.6 ± 1.3	6.6 ± 0.5
U937	43.5 ± 0.2**	36.2 ± 3.3**	13.4 ± 0.8**	10.6 ± 2.8**	1.4 ± 0.0	1.4 ± 0.0
RAJI	63.4 ± 0.9**	52.3 ± 1.2**	49.1 ± 0.2**	40.8 ± 1.1**	4.4 ± 0.0	4.1 ± 0.3

The means of percentages of cells in the sub-G1 phase followed by an *asterisk* (*) indicate statistically significant difference from the DMSO-treated control at $P < 0.05$. "**" means statistically significant difference from the DMSO-treated control at $P < 0.01$.

[a] Untreated control

[b] DMSO-treated control

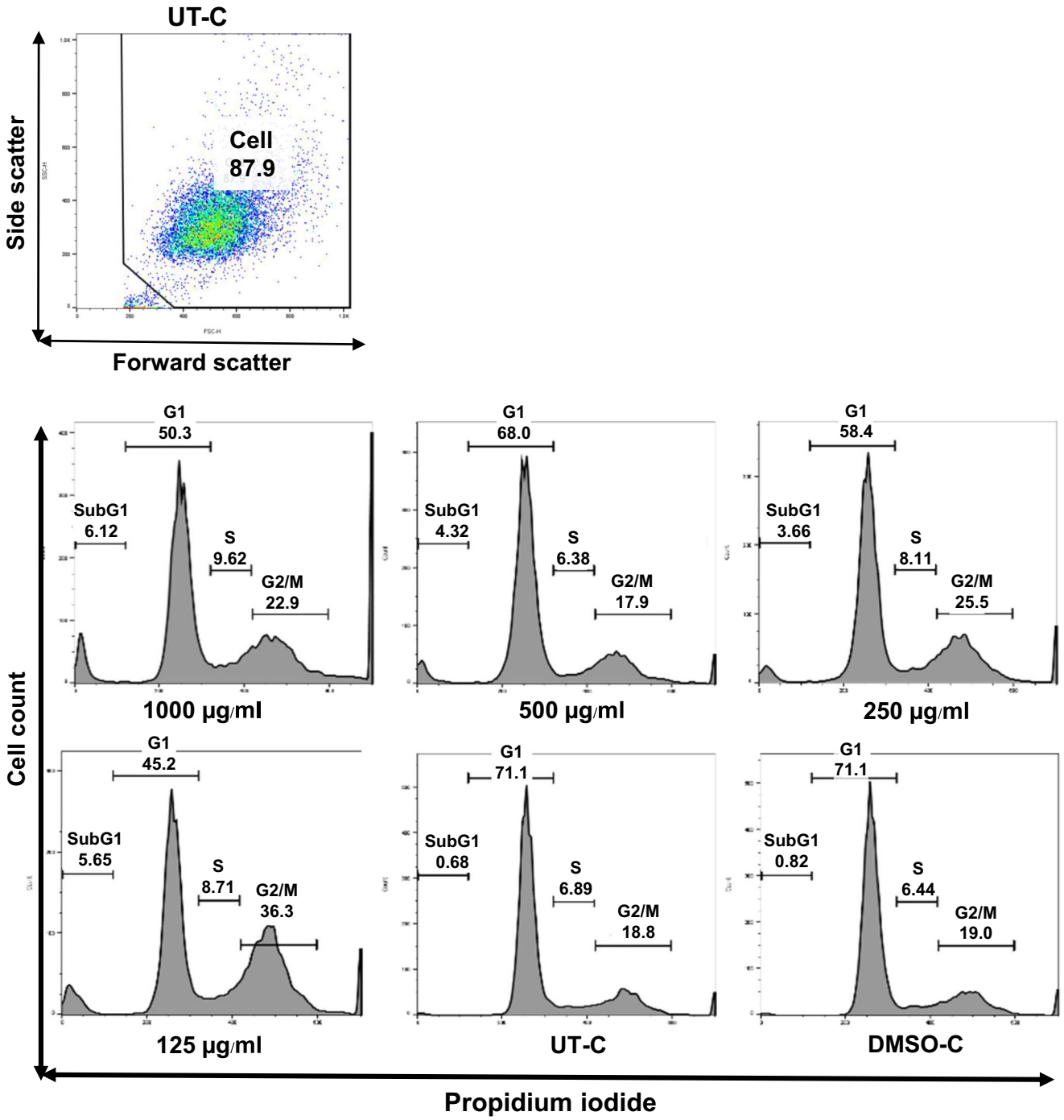

Figure 2. The cell cycle analysis of SiHa cells treated with 125–1000 µg/ml of extract obtained from the stems of *U. longipes* (SUL). Untreated (UT-C) cells and DMSO were used as the controls. (*A*) Shows the gating strategy. (*B*) Histograms show the patterns of cells stained with PI.

staining and analyzed by flow cytometry, as described previously (Pumiputavon *et al.* 2017).

Cell cycle analysis The cell cycle was assessed by staining human cancer cell lines with 20 µg/ml PI and analyzed by flow cytometry, as described previously (Pumiputavon *et al.* 2017).

Phytochemical screening All the extracts were phytochemical-tested for alkaloids, sterols, cardiac glycosides, anthraquinone glycosides, saponins, flavonoids, and tannins by using standard methods, as described previously (Mujeeb *et al.* 2014). The results are expressed qualitatively as (−) for the absence; and (+), (++), and (+++) for the presence

Table 4 Phytochemical constituents of methanolic stem-derived extract

Phytochemical	*U. longipes*	*Dasymaschalon* sp.	*A. burmanicus*	*M. modestum*
Alkaloids	++	++	++	++
Sterols	+	+	+	+
Tannins	++	++	+	+
Saponins	–	–	–	–
Anthraquinone glycosides	++	++	++	++
Flavonoids	++	++	++	+
Coumarins	+++	+++	++	–

of low, medium, and high content of phytochemicals, respectively.

Evaluation of acetogenins The extracts were analyzed for five acetogenins, including annoglaxin, squamostatin-A, bullatacin, squamocin, and murisolin by a reversed-phase HPLC (RP-HPLC) system (Agilent 1200 Series, Santa Clara, CA) with slight modifications from Yang *et al.* (2010). Separation was accomplished on a reversed-phase C18 column (150 × 4.6 mm inner diameter) (KINETEX® C18; Phenomenex Co., Ltd., Torrance, CA). The mobile phases consisted of A (methanol) and B (deionized water) using linear gradients of 0–40 min (85%A) and 40–60 min (85–95%A). The mobile phase pumped at a flow rate of 0.3 mL/min and the detection wavelength was 220 nm. Series of corresponding standards were measured concomitantly to construct a calibration curve used for determination of the concentrations of annoglaxin, bullatacin, squamocin, asiminecin, and murisolin in samples. The assays were performed in triplicate.

Statistical analysis Statistical analysis was performed using SPSS program. One-way ANOVA with Tukey's HSD *post hoc* test was used to determine differences between groups. Significance was set at $P < 0.05$.

Results

Induction of cancer cell death by stem-derived extracts from four species of the *Annonaceae* family The percentages of cell death determined by flow cytometry following Annexin V and PI staining were used to evaluate the cytotoxic activity of the extracts. The populations of cells in the Annexin V+/PI− and the Annexin V+/PI+ quadrants were identified as induced cell death. The half-maximal inhibitory concentration (IC_{50}) was calculated from the percentages of cell death (Annexin V+/PI−, Annexin V−/PI+, and the Annexin V+/PI+ quadrants) (Table 1). The degree of apoptosis varied by type of extract (stem from *Uvaria longipes*: SUL, *Dasymaschalon* sp.: SD, *Artabotrys burmanicus*: SAB, and *Marsypopetalum modestum*: SMM). SUL presented the best cytotoxic activity among the extracts, especially on hepatocellular carcinoma lines (HepG2 and Hep3B). Morphological changes were observed using an inverted microscope together with the decreases of the mean forward light scatter from flow cytometry (data not shown). These changes were observed in the extracts that had cytotoxic activity and in only the cell lines that were affected. The affected cell lines had shrunken cytoplasm, condensed chromatin, and loss of normal shape. SAB and SMM showed least cytotoxic activity on almost all cell lines even at 500 and 1000 μg/ml concentrations (Table 2). The effect of SD was prominent only on HepG2 cell line. Human myeloid leukemia lines (K562, U937, and RAJI) were less affected by all the extracts compared with cervical carcinoma and human hepatocellular carcinoma groups. These indicate that the extracts induced cytotoxicity in a cell-type specific manner. In addition, a dose-dependent manner was also observed in some extracts (Table 2 and Fig. 1).

Effect of SUL on cell cycle arrest From apoptosis assessment, it was evident that SUL showed the highest cytotoxic activity on most of the cancer cell lines. Therefore, SUL was further evaluated for its effect on cell cycle. After treating the cancer cell lines with various concentrations of SUL for 24 h, the cell cycle was assessed by propidium iodide staining followed by flow cytometry. SUL induced cell cycle arrest of all the cancer cell lines, except Hep3B, in a dose-dependent manner as evidenced by the increase of the sub G1-phase population compared with the controls (untreated or DMSO-treated cells) (Table 3 and Fig. 2).

Figure 3. The HPLC chromatograms of the reference compounds and four crude methanolic stem-derived extracts. A mixture of five acetogenins including annoglaxin (*1*), bullatacin (*2*), squamocin (*3*), asiminecin (*4*), and murisolin (*5*); and crude methanolic stem-derived extract of *U. longipes*; *Dasymaschalon* sp.; *A. burmanicus*; and *M. modestum*.

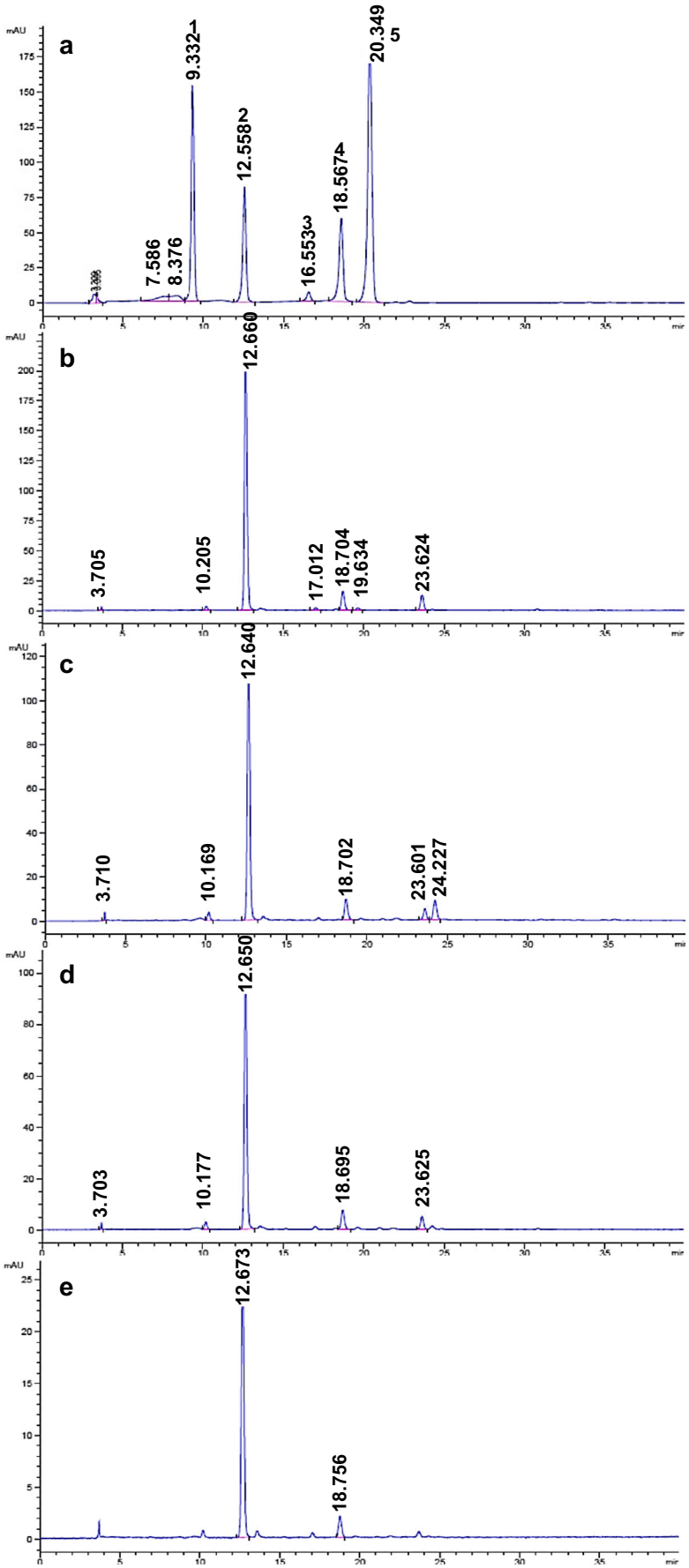
a
9.332
12.558
16.553
18.567
20.349
7.586
8.376
b
12.660
3.705
10.205
17.012
18.704
19.634
23.624
c
12.640
3.710
10.169
18.702
23.601
24.227
d
12.650
3.703
10.177
18.695
23.625
e
12.673
18.756

Table 5 Contents of five acetogenins in four methanolic stem-derived extracts (mg/g extract)

Acetogenin	*U. longipes*	*Dasymaschalon sp.*	*A. burmanicus*	*M. modestum*
Annoglaxin	nd	nd	nd	nd
Bullatacin	0.86 ± 0.04^{a}	0.63 ± 0.05^{b}	0.55 ± 0.07^{bc}	0.38 ± 0.06^{d}
Squamocin	nd	nd	nd	nd
Asiminecin	0.39 ± 0.05^{A}	0.35 ± 0.06^{AB}	0.28 ± 0.04^{BC}	0.18 ± 0.04^{D}
Murisolin	nd	nd	nd	nd

"nd" = not detected

"A" or "a" = extract obtained from the stem of *U. longipes*

"B" or "b" = extract obtained from the stem of *Dasymaschalon* sp.

"C" or "c" = extract obtained from the stem of *A. burmanicus*

"D" or "d" = extract obtained from the stem of *M. modestum*

Significant differences in the amount of each active compound between extracts is indicated by "a," "b," "c," or "d" for bullatacin; or "A," "B," "C," or "D" for asiminecin. The attached superscripts demonstrate the amount of active compound considered as regards statistical differences (*P* value lower 0.05) between the corresponding.

Phytochemical analysis Preliminary phytochemical screening demonstrated that all the extracts contained alkaloids, coumarins, sterols, tannins, anthraquinone, glycosides, and flavonoids. The contents of the phytochemicals are shown in Table 4. Saponins were not found in any extracts. All the extracts were analyzed for acetogenins by reversed-phase HPLC, and compared with five standard acetogenins, including annoglaxin, squamostatin-A, bullatacin, squamocin, and murisolin. The HPLC chromatogram (Fig. 3) revealed that all the extracts contained bullatacin and asiminecin (Table 5).

Discussion

The *Annonaceae* family has been recognized as a source of very rich secondary metabolites, including terpenoids, alkaloids, steroids, polyphenols, and flavonoids (Moreira *et al.* 2013). In the present study, the cytotoxic and cytostatic activities of methanolic extracts of stems of these four species against human cervical carcinoma (HeLa and Caski), human hepatocellular carcinoma (HepG2 and Hep3B), and human myeloid leukemia (K562, U937, and RAJI) cancer cell lines were evaluated. The results showed that the extracts induced cell death in a dose-dependent and cell-type specific manner. The extract obtained from the stem of *U. longipes* induced the accumulation of the SubG1 phase of some cancer cell lines, also in a dose-dependent and cell-type specific manner.

The phytochemical analysis showed the presence of alkaloids, coumarins, sterols, tannins, anthraquinone glycosides, and flavonoids in all the extracts. Alkaloids have several therapeutic properties such as anti-inflammatory, hepatoprotective, and anti-cancer activities (Takshak 2018). Coumarins regulate a number of cellular pathways and have been reported as showing anti-cancer activity on different types of cancers (Thakur *et al.* 2015). Tannins exhibit potential anti-cancer effect *in vitro*, including triggering apoptosis and cell cycle arrest, inhibition of invasion and metastases, and inhibiting angiogenesis (Cai *et al.* 2017), though further study *in vivo* is needed. Sterol has been proposed as a possible therapeutic agent against cancer by inhibition of various regulatory molecules in the cholesterol homeostasis of cancer cells (Gabitova *et al.* 2014). The activities of flavonoids on cancer cells include anti-proliferative effect, inhibiting tumor formation, promoting apoptosis, and inhibiting the activation of nuclear factor kappa B (NF-κB) signaling pathway (Tungmunnithum *et al.* 2018). All the extracts in our study contained alkaloids, coumarins, sterols, tannins, anthraquinone glycosides, and flavonoids. An exception was in the case of coumarins, which were not detected in the stem-derived methanolic extracts of *M. modestum*. The lack of flavonoids in *M. modestum* is consistent with the lowest cytotoxic activity of this extract as compared with the others.

Bullatacin and asiminecin were detected in all the extracts by HPLC. These two active compounds have been reported for various biological activities. Bullatacin isolated from an Annonaceae plant is capable of inducing apoptosis of human hepatoma cell line through a mechanism that reduces intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP) levels (Chiu *et al.* 2003). An *in vivo* study showed that treatment of mice with bullatacin results in reduction in tumor growth with improved hematologic parameters (Chen *et al.* 2013). Moreover, bullatacin can induce apoptosis of human cervical cancer HeLa and human leukemia HL-60 cells by inhibition of NADH oxidase activity (Morre *et al.* 1995). Asiminecin has shown promising cytotoxicity and exhibiting potential for development as a potential anti-cancer agent (Mangal *et al.* 2015). The cytotoxic activity has been demonstrated on the HT-29 human colon cancer cell line (Zhao *et al.* 1994). However promising these results may seem, they are just preliminary findings. All the extracts should be tested for

cytotoxicity against normal cells, such as human mononuclear cells. It is also worth noting that the fruits, seeds, and leaves of the Annonaceae family contain a neurotoxic compound called annonacin which is possibly responsible for atypical Parkinsonism/dementia clusters (Smith *et al.* 2016). It has been reported that the stem of the Annonaceae family contains annonacin which is cytotoxic and insecticidal and inhibited the formation of crown gall tumors on potato discs (Alkofahi *et al.* 1988). Whether the Annonaceae plants tested in our study contain annonacin needs further investigation. Phytochemical analysis and the cytotoxicity tests would guarantee the suitability and safety of these extracts in therapeutic application against different cancer cell lines in the future.

Conclusions

Stem-derived methanolic extracts of *U. longipes*, *Dasymaschalon* sp., *A. burmanicus*, and *M. modestum* exhibited evidence of cell death induction in a cell-type specific manner. The extract of *U. longipes* showed the highest cytotoxic activity. Cancer cell lines treated with this extract showed increased percentage of cells in SubG1 phase of the cell cycle. All the extracts in this study offer a potential source of bullatacin and asiminecin, which might be an alternative or complementary remedy for cancer management.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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