Journal of Medicinal Plants Research Vol. 6(21), pp. 3711-3723, 9 June, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.599
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Anti-proliferation and anti-migration activities of ten selected *Zingiberaceae* species against MDA-MB-231 cells

Ann Jee Chew[1]*, Nurhayati Zainal Abidin[1] and Norhanom Binti Abdul Wahab[2]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Center for Foundation Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Accepted 30 August, 2011

***Zingiberaceae* plants exhibit various biological activities, but scientific knowledge of the plants in anti-metastatic aspects is still very limited. The objective of this study is to examine the anti-metastatic potentials of 30 crude extracts from ten selected local *Zingiberaceae* species on hormone-independent, highly metastatic human breast cancer cells, MDA-MB-231. The *Zingiberaceae* rhizomes were extracted with petroleum ether, chloroform, and methanol using Soxhlet extractor system. Effects of the 30 extracts on proliferation and migration of MDA-MB-231 cells were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and scratch wound assay, respectively. Besides cytotoxicity, it is also vital to determine the potential toxicity of the extract in order to select the most promising extracts for anti-cancer drugs development. Thus, a special parameter–Selectivity Index (SX) was utilized as the selectivity indicator of tested extracts towards tumour cells. The results revealed that petroleum ether extracts of *Alpinia galanga*, *Boesenbergia rotunda*, and *Zingiber zerumbet*; as well as chloroform extracts of *Alpinia galanga*, *Boesenbergia rotunda*, and *Curcuma domestica* were screened to possess the most effective anti-proliferation and anti-migration activities against MDA-MB-231 cells. Three most desirable extracts were selected based on both their anti-metastatic potentials and SX, and were subjected for qualitative analysis using thin-layer chromatography (TLC).**

Key words: *Zingiberaceae*, anti-metastatic activity, anti-migration, anti-proliferation, MDA-MB-231, selectivity index.

INTRODUCTION

Cancer is an ailment in which cells escape from the body's normal regulatory mechanisms and behave ectopically (Laundry de Mesquita et al., 2009). There are several stages in carcinogenesis, known as initiation, promotion and progression stages. In the initiation stage, mutations occur and not corrected by the immune cellular mechanism resulting in the formation of cancer cells. In the promotion stage, cancer cell proliferate uncontrollably and transformed into malignant cell. Then, malignant cell transformed into malignant tumour in the progression stage. Cancer can be cured successfully by surgical removal of the tumours if the malignant cells are to remain in a single mass. However, malignant tumours are capable of detaching from the primary tumour mass, entering the blood stream or lymphatic channels, followed by localization and growth of secondary tumours at the new sites of other body's parts (Hayot et al., 2006). The dispersion of tumour within the body is known as metastasis, which significantly results in morbidity and mortality, thus causing cancer a devastating disease.

Breast cancer accounts for approximately 20% mortality in the world, and 76% of all breast tumours have been categorized as invasive breast cancers. Breast tumours are capable of metastasise to local lymph nodes as well as to organs such as bone, lung and liver (Shanmugaraj et al., 2010). Current treatments for the

*Corresponding author. E-mail: c_annjee@yahoo.com.

pathology include excision surgery, chemotherapy, radiotherapy, as well as frequently supported by adjuvant chemo- or hormone-therapies (Lin et al., 2009; Tsai et al., 2007). These therapies kill a large number of normal cells along with the cancer cells due to their low specificity. Besides that, the problem of drug resistance in breast cancer has been the major obstacle in chemotherapeutic treatment (Tannin-Spitz et al., 2007). It is also a painfully evident that chemotherapy and radiation cause severe adverse effects, such as bone marrow suppression resulting in cytopoenia, and subsequent devastation of the immune responses (Devasagayam and Sainis, 2002), in addition to exhibit limited curative value for most advance cancers. Based on these scenarios, agents that can inhibit the metastatic activity of the cancer cells are considered as a promising cancer treatment.

In the global scenario, low prevalence of breast cancer in Asian countries as compared to Western countries is due to their dietary preferences, such as Soy, Turmeric, etc. (Fotsis et al., 1995). Herbs possess various pharmacological activities including antioxidant and anti-inflammatory effects which are associated with anti-mutagenic and anti-carcinogenic properties (Bode et al., 2001). In Malaysia, rhizomes of Zingiberaceae plants as herbal medicines have been practiced long since historical time due to their health-promoting effects. The rhizomes are utilized for various ailments such as stomache, diarrhea, digestive disorders, rheumatism, swelling, common cold and cough (Yap et al., 2007). Many researches on potential compounds of the *Zingiberaceae* rhizomes in anti-inflammatory, anti-oxidative, and anti-mutagenic properties have been studied (Surh, 2002, 1999; Surh et al., 1998). However, scientific knowledge concerning the *Zingiberaceae* rhizomes in anti-metastatic aspects is still very limited.

Considering the reported strong correlation between dietary factors and cancer prevention (Rogers et al., 1993; Surh, 2003) and the potential biological activities of Zingiberaceae plants, evidence exists showing the possible effects of local *Zingiberaceae* species on cancer metastasis. Therefore, the objective of this study was to examine the effects of 30 crude extracts from ten selected *Zingiberaceae* species on the proliferation and migration of highly metastatic human breast cancer cells, MDA-MB-231. The employed of MDA-MB-231 cells in this study, as the properties of these cells are akin to human breast cancer cells in addition to its hormone-independent characteristic. In the present study, toxicity of the most cytotoxic extracts would be determine to better select the most desirable extracts for further investigation.

MATERIALS AND METHODS

Plant materials

Ten local medicinal plants in the Zingiberaceae family, namely *Alpinia galanga*, *Boesenbergia rotunda*, *Curcuma aeruginosa*, *Curcuma domestica*, *Curcuma mangga*, *Curcuma xanthorrhiza*, *Kaempferia galanga*, *Zingiber montanum*, *Zingiber officinale*, and *Zingiber zerumbet* were selected for *in vitro* screening tests. Fresh rhizomes of the selected *Zingiberaceae* species were bought from Faridah Bte. Zakariah (001302008-V), No: 424/348 located at Chow Kit Market, Kuala Lumpur, Malaysia.

Preparation of crude extracts

Fresh *Zingiberaceae* rhizomes were washed with water, cut into small pieces and dried in an oven at 50°C for three days. Dried rhizomes of each species were grounded into fine powder, and extracted with petroleum ether, chloroform and methanol using conventional Soxhlet extractor system (Houghton and Raman, 1998). A total of 30 crude *Zingiberaceae* extracts were obtained for the *in vitro* screening test.

Cell culture

MDA-MB-231 (estrogen negative) cell line was obtained from the American Type Culture Collection (Rockville, USA). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, USA) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (PAA Laboratories GmbH, Australia), 2% penicillin/ streptomycin (PAA Laboratories GmbH, Australia), and 1% amphotericin B (PAA Laboratories, GmbH, Australia). Cells were maintained at 37°C in a humidified incubator of 5% carbon dioxide (CO_2) and 95% air.

Cell proliferation and viability assay

Effects of the *Zingiberaceae* extracts on proliferation and viability of MDA-MB-231 cells were measured using MTT (Sigma, USA) assay. Growth of the cancer cells was quantified by the ability of viable cells to reduce the yellow dye MTT to a blue formazan product (Mossmann, 1983). Cells were seeded at a density of 8×10^3 cells/well, in 100 µl DMEM of 10% FBS, in 96-well plates (Nunc, Denmark) for 24 h.

Then, the cells were treated with serially diluted *Zingiberaceae* extracts dissolved in 0.1% dimethyl sulphoxide (DMSO) (Sigma, USA) at 1.56 to 100 µg/ml for 48 h. Control groups included untreated cells or negative control, and doxorubicin hydrochloride (Sigma, USA) (1.20 to 12.5 µg/ml) or positive control, received the same amount of DMSO. After 48 h, the medium in each well was replaced with fresh medium (100 µl), and 20 ul of 5 mg/ml MTT in phosphate buffer saline (PBS) pH 7.2, and incubated for 4 h. After 4 h, the formazan products formed at the bottom of each well were dissolved in 100 µl DMSO. The optical density at 540 nm (OD_{540}) was measured using a microplate reader (Emax Precision, Molecular Devices).

In vitro toxicity assay

The most cytotoxic extracts against MDA-MB-231 cells were selected for *in vitro* toxicity study in order to assess their potential toxicity against normal, non-tumoural cells, MRC-5. Cytotoxicity of the extracts against MRC-5 cells (8×10^3 cells/well, in 100 µl MEM of 10% FBS in 96-well plates) was performed using MTT assay for 48 h. A special parameter–Selectivity Index (SX) was applied as a selectivity indicator of tested substances towards tumour cells (Popiolkiewicz et al., 2005).

In this context, the SX represents the ratio of IC_{50} obtained in toxicity testing using MRC-5 cells to IC_{50} of the same extract in cytotoxicity test using MDA-MB-231 cells, and then multiplied by

100 as represented by the equation:

$$SX = \frac{IC_{50\ (MRC\text{-}5)}}{IC_{50\ (MDA\text{-}MB\text{-}231)}} \times 100 \quad (1)$$

Scratch wound assay

MDA-MB-231 cells were seeded in a six-well plates (Nunc, Denmark) at 1 × 10^6 cells/well and cultured in DMEM containing 10% FBS for 24 h. Confluence cells were scrapped using 200 µl pipette tip along the diameter of the well, and cell debris was discarded by washing with PBS. The wounded monolayer was incubated in the absence (negative control) and presence of *Zingiberaceae* extract (dissolved in 0.1% DMSO) in DMEM containing 1% FBS. [6]-Gingerol (Sigma, USA) (positive control) was used as the positive control. Wound closure of the cells was observed under the microscope at magnification 50×, and photographs were captured immediately after treatment (0 h), 24 h, and 48 h later. Five fields of the wounds were captured and mean values for the selected five fields pre well were analysed. A special parameter–absolute migration capability (MC_A) was applied to measure the migration capability of MDA-MB-231 cells after treated with the extracts. Although the relative cell migration capability has been described by Liu et al. (2005) and Peng et al. (2006), it has been modified by Peng et al. (2007) to absolute migration capability (MC_A) as:

$$MC_A\ (mm/h) = \frac{G_0 - G_t}{2t} \quad (2)$$

Where G_0 is the initial gap for the cell line at 0 h (mm), G_t is the final gap for the cell line treated with *Zingiberaceae* extract at certain concentration or vehicle alone for a time period of t (h) (mm), and t is the overall time period for incubation (h).

Thin-layer chromatography

The most potential extracts screened were selected for qualitative analysis using TLC to examine the chemical groups contained in the crude extracts. Choice of detection methods included examination under daylight, UV light at 254 nm, UV light at 365 nm, exposure to iodine vapour, and the use of chromogenic sprays such as 50% sulphuric acid (H_2SO_4) reagent, Dragendorff's reagent, and vanillin/H_2SO_4 reagent.

Statistical analysis

All experiments were performed at least three times (N = 3). All quantitative data were presented as mean ± standard error of mean (S.E.M) and analysed using the Statistical Package for the Social Sciences (SPSS) Version 16.0 and Graphpad Prism 4.02 programme.

For cell proliferation and viability assay and *in vitro* toxicity assay, cytotoxicity of the extracts against the cells was presented in term of Cytostatic concentrations (IC_{50}). In this context, IC_{50} were identified as the concentration of *Zingiberaceae* extract required to inhibit 50% cell proliferation or viability as compared to the negative control. The IC_{50} values were obtained by nonlinear regression using the Grahpad Prism Version 4.02 programme. For scratch wound assay, gap distances were compared and analysed via Paired-Samples *t*-test. P values < 0.05 were considered statistically significant.

RESULTS

Effects of the *Zingiberaceae* extracts on MDA-MB-231 cell proliferation and viability

Complete dose-response curves were generated and IC_{50} values (Table 1) were calculated for the *Zingiberaceae* extracts against MDA-MB-231 cells. Based on Table 1, 12 extracts showed IC_{50} lower than 30 µg/ml, nine of them showed IC_{50} lower than 20 µg/ml, and among them, four with IC_{50} lower than 10 µg/ml. These extracts with IC_{50} lower than 30 µg/ml were petroleum ether extracts of *A. galanga*, *B. rotunda*, *C. domestica*, *Z. montanum*, and *Z. zerumbet*; chloroform extracts of *A. galanga*, *B. rotunda*, *C. domestica*, *C. xanthorrhiza*, *K. galanga*, and *Z. montanum*; as well as methanol extract of *C. domestica*. The strongest cytotoxicity activity was detected for the petroleum ether extract of *A. galanga*, which presenting the lowest IC_{50} value, 2.82 µg/ml.

The results demonstrated that no appreciable loss in cell growth was observed in cells incubated with increased concentrations of methanol extracts of *A. galanga*, *C. aeruginosa*, and *C. mangga*, *Z. montanum*, *Z. officinale* and *Z. zerumbet*; as well as petroleum ether extracts of *C. mangga* and *K. galanga*. These extracts possessed IC_{50} values which were more than 100 µg/ml. The cytotoxicity of the extracts were evaluated using MTT assay. Cells were plated in 96-well microplates and treated with various concentration of the *Zingiberaceae* extracts for 48 h. Then, the medium in each well was replaced with 100 µl fresh media, and 20 µl of MTT dye solution, and further incubated for 4 h. After that, 100 µl of DMSO was added to solubilize the formazan products formed in each well. Absorbance at 540 nm was measured using a microplate reader as a measure of the density of viable cells. Results shown are the mean of three-independent experiments with triplicates treatments.

Cytotoxicity of the *Zingiberaceae* extracts on MRC-5 cells

Six most active *Zingiberaceae* extracts which exhibited significant inhibitory effect on the proliferation and viability of MDA-MB-231 cells (IC_{50} value ≤ 10 µg/ml) (Table 1) were selected for *in vitro* toxicity study against normal, non-tumoural human lung fibroblast cells, MRC-5 using MTT assay for 48 h. In the selection of the least toxic extracts among the most active ones, a special parameter, known as Selectivity Index (SX) was applied. Selectivity Index is a selectivity indicator of tested substances towards tumour cells.

In this experiment, the SX represents the ratio of IC_{50} obtained in toxicity testing using MRC-5 cells to IC_{50} of the same extract in cytotoxicity test using MDA-MB-231 cells, and then multiplied by 100 ($SX = IC_{50(MRC\text{-}5)}/IC_{50(MDA\text{-}MB\text{-}231)} \times 100$). The SX value above 100 indicates that the

Table 1. *In vitro* cytotoxicity of the *Zingiberaceae* extracts on MDA-MB-231 cells measured using MTT assay.

***Zingiberaceae* species**[1]	**Solvent**[2]	**IC_{50} (µg/ml) MDA-MB-231 cell line**[3]
	P	2.87
Alpinia galangal	C	6.19
	M	>100
	P	9.21
Boesenbergia rotunda	C	10.25
	M	55.06
	P	30.13
Curcuma aeruginosa	C	55.68
	M	>100
	P	14.34
Curcuma domestica	C	10.10
	M	15.51
	P	>100
Curcuma mangga	C	30.10
	M	>100
	P	42.78
Curcuma xanthorrhiza	C	17.34
	M	58.78
	P	>100
Kaempferia galangal	C	29.63
	M	48.76
	P	26.16
Zingiber montanum	C	25.38
	M	>100
	P	55.93
Zingiber officinale	C	30.06
	M	>100
	P	7.46
Zingiber zerumbet	C	31.48
	M	>100
Doxorubicin hydrochloride	-	1.27

[1] Ten selected *Zingiberaceae* rhizomes were selected for *in vitro* cytotoxicity study using MTT assay. [2] Solvents used in extraction: p: petroleum ether, c: chloroform, m: methanol; (-) not tested. [3] MDA-MB-231 (human breast cancer cell line, oestrogen-receptor negative).

cytotoxic effect of tested substance is greater towards cancer cells. The SX value of 100 or below would suggest that the tested concentration of the substance for achieving therapeutic effect is similar to or lower than the concentration causing toxic effects to normal cells. Therefore, the most promising substances for development of anti-cancer drug would have SX values distinctly higher than 100.

Table 2. *In vitro* cytotoxicity of the *Zingiberaceae* extracts on MRC-5 and MDA-MB-231 cells and their selectivity index.

Zingiberaceae species[1]	Solvent[2]	Cell line[3], IC_{50} (µg/ml)		Selectivity index[4] (SX)
		MRC-5	MDA-MB-231	
Alpinia galanga	P	2.86	2.87	100
	C	11.87	6.19	192
Boesenbergia rotunda	P	12.78	9.21	139
	C	13.22	10.25	129
Curcuma domestica	C	12.32	10.10	122
Zingiber zerumbet	P	12.09	7.46	162

[1]Six most active *Zingiberaceae* extracts with $IC_{50} \leq 10$ µg/ml in the cell proliferation and viability assay were selected for *in vitro* cytotoxicity against MRC-5 cells using MTT assay. [2]Solvents used in extraction: p: petroleum ether, c: chloroform, m: methanol. [3]MRC-5 (human lung fibroblast cell line), MDA-MB-231 (human breast cancer cell line, oestrogen-receptor negative). [4]SX: Selectivity Index calculated by Equation 1.

The results (Table 2) revealed that only crude petroleum ether extract of *A. galanga* possessed SX value equal to 100. This indicated that the cytotoxicity of this extract to kill cancerous cells (MDA-MB-231 cells) and non-cancerous cell (MRC-5) were similar, and thus it was inappropriate for further studies even though it is the most cytotoxic extract against MDA-MB-231 cells (Table 1). The other tested extracts possess SX values distinctly higher than 100, which indicated that their cytotoxicity is more prone to kill cancerous cells (MDA-MB-231) than normal cells (MRC-5), and thus were promising for further studies in the development of anti-cancer drug.

The cytotoxicity effects of the extracts were evaluated using MTT assay. Cells were plated in 96-well micro-plates and treated with various concentration of the *Zingiberaceae* extracts for 48 h. Then, the medium in each well was replaced with 100 µl fresh media, and 20 µl of MTT dye solution, and further incubated for 4 h. After that, 100 µl of DMSO was added to solubilize the formazan products formed in each well. Absorbance at 540 nm was measured using a microplate reader as a measure of the density of viable cells. Results shown are the mean of three-independent experiments with triplicates treatments.

Effects of the Zingiberaceae extracts on migration of MDA-MB-231 cells

Cell migration is an important factor to probe metastatic potentials of cancer cells. The anti-migratory activities of the *Zingiberaceae* extracts against MDA-MB-231 cells were examined using Scratch wound assay. This study was conducted at the categorized concentrations of the *Zingiberaceae* extracts based on their IC_{50} values (Table 1). The extracts were classified into four categories according to the effectiveness of their anti-proliferative potentials. Inhibitory effects of the *Zingiberaceae* extracts on the proliferation and viability of MDA-MB-231 cells were classified into four categories, namely strongly active (IC_{50} < 30 µg/ml), moderately active (IC_{50}, 30 to 55 µg/ml), weakly active (IC_{50}, 56 to 100 µg/ml), and inactive (IC_{50} > 100 µg/ml) (Table 3). Anti-migratory activity of the extracts in strongly active, moderately active, weakly active and inactive categories was conducted at 12.5, 25, 50, and 100 µg/ml, respectively.

Absolute migration capability (MC_A) was used as the parameter for evaluating migration capability of MDA-MB-231 cells after treated with crude *Zingiberaceae* extracts *in vitro*. MC_A value for untreated cells (negative control) was 6.03×10^{-3} mm/h at 48 h of incubation time, which represented the migration capability of MDA-MB-231 cells in normal conditions. Untreated cells were able to invade the scratched area that is fully re-colonised at the end of 48 h incubation. In the Scratch wound assay, the largest gaps observed after treated with the extracts revealed the most effective agent in anti-migration. The MDA-MB-231 cells treated with *Zingiberaceae* extracts spreading along the wound edges would have become slower than that of cells with vehicle alone. In comparison with the MC_A values (Table 4), all of the tested *Zingiberaceae* extracts except crude methanol extract of *C. aeruginosa* (tested at 100 µg/ml) did inhibit or slow down the migration activity of MDA-MB-231 cells.

The smaller the value of MC_A manifests the lower migration capability. Negative MC_A values represented no migration activity could be observed. Based on the results in Table 4, petroleum ether and chloroform extracts of *A. galanga* (tested at 12.5 µg/ml) were showed to be the most active extracts with MC_A values -5.73×10^{-3} and -8.02×10^{-3} mm/h at 48 h of incubation, respectively. The anti-migration effects may accompany by some level the extracts' cytotoxicity. Besides that, chloroform extract of *C. domestica* (tested at 12.5 µg/ml), and petroleum ether extract of *Z. zerumbet* (tested at 12.5 µg/ml) were showed to be very effective in inhibiting the migration of MDA-MB-231 cells, with MC_A values 1.35×10^{-4} and 1.63×10^{-3} mm/h at 48 h of incubation, respectively.

It was interesting to find that most of the methanol extracts of *Zingiberaceae* which were inactive in anti-proliferation activities, exerted anti-migration activities at

Table 3. Classifications of the *Zingiberaceae* extracts according to the effectiveness of their anti-proliferation activity against MDA-MB-231 cells.

Strongly active (IC_{50} < 30 µg/ml)	Moderately active (IC_{50}, 30–55 µg/ml)	Weakly active (IC_{50}, 56–100 µg/ml)	Inactive (IC_{50} > 100 µg/ml)
Alpinia galanga (p)	*Boesenbergia rotunda* (m)	*Curcuma aeruginosa* (c)	*Alpinia galanga* (m)
Alpinia galanga (c)	*Curcuma aeruginosa* (p)	*Curcuma xanthorrhiza* (m)	*Curcuma aeruginosa* (m)
Boesenbergia rotunda (p)	*Curcuma mangga* (c)		*Curcuma mangga* (p)
Boesenbergia rotunda (c)	*Curcuma xanthorrhiza* (p)		*Curcuma mangga* (m)
Curcuma domestica (p)	*Kaempferia galanga* (c)		*Kaempferia galanga* (p)
Curcuma domestica (c)	*Kaempferia galanga* (m)		*Zingiber montanum* (m)
Curcuma domestica (m)	*Zingiber montanum* (p)		*Zingiber officinale* (m)
Curcuma xanthorrhiza (c)	*Zingiber montanum* (c)		*Zingiber zerumbet* (m)
Zingiber zerumbet (p)	*Zingiber officinale* (p)		
	Zingiber officinale (c)		
	Zingiber zerumbet (c)		

[1]Each crude *Zingiberaceae* extract was tested at 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/ml in triplicates (N = 3). The extracts were tested against MDA-MB-231 cells using MTT assay. The effectiveness of the anti-proliferation activity was categorized into four groups, namely strongly active, moderately active, weakly active, and inactive according to their IC_{50} values. In the Table, p represents petroleum ether extract, c represents chloroform extract, and m represents methanol extract.

the end of 48 h incubation. These extracts are methanol extracts of *A. galanga* (MC_A, 5.78 × 10^{-3} mm/h), *C. mangga* (MC_A, 5.72 × 10^{-3} mm/h), *Z. montanum* (MC_A, 4.43 × 10^{-3} mm/h), *Z. officinale* (MC_A, 4.81 × 10^{-3} mm/h) , and *Z. zerumbet* (MC_A, 4.45 × 10^{-3} mm/h). In the comparisons of MCA values between 24 and 48 h of incubation time, anti-migration effects of some extracts could only be seen at the end of 48 h incubation. These extracts were methanol extract of *A. galanga* (tested at 100 µg/ml), petroleum ether extract of *C. xanthorrhiza* (tested at 25 µg/ml), and methanol extract of *K. galanga* (tested at 25 µg/ml). Based on the scenarios, Scratch wound assay is much better to be performed at 48 h time point rather than just 24 h, which could better elaborate the anti-migration acitivities of each crude *Zingiberaceae* extracts.

Effects of the extracts on migration of MDA-MB-231 cells were determined using scratch wound assay. Cells were plated in six-well plate and treated with *Zingiberaceae* extracts at their categorized concentrations. Photographs were taken at 0, 24 and 48 h, and the gap distances were measured using a computerized phase-contrast microscope. Results shown are mean of three-independent experiments with duplicates treatments.

Qualitative analysis using thin-layer chromatography

Three most potential *Zingiberaceae* tested based on the screening tests were chloroform extract of *A. galanga*, chloroform extract of *C. domestica*, and petroleum ether extract of *Z. zerumbet*. These extracts showed potent anti-proliferation and anti-migration activities against human breast cancer cells, MDA-MB-231. More importantly, these extracts possess SX values distinctly greater than 100 (Table 2), which indicated their promising properties for further studies in the development of anti-cancer drugs.

The extracts were subjected for qualitative analysis using TLC to determine a large extent of nature substances present in the crude extracts. Types of compounds detected from the TLC tests for crude chloroform extract of *A. galanga*, crude chloroform extract of *Curcuma domestica*, and crude petroleum ether extract of *Z. zerumbet* were presented in Tables 5, 6 and 7, respectively. These may already be known as the results of preliminary chemical tests.

Based on Table 5, 15 compounds were detected in the crude chloroform extract of *A. galanga*. Nine (60%) of the compounds containing conjugated double bonds, or compounds with double bond. Twelve compounds were detected in thee crude chloroform extract of *C. domestica* (Table 6). It was interesting to observed that C_6 (R_f = 0.66) showed positive results for all of the detection methods applied. Eleven (92%) of the compounds containing conjugated double bonds and eight (67%) were organic compounds. For crude petroleum ether extract of *Z. zerumbet*, 15 compounds were detected (Table 7). Seven (47%) of the compounds were from the group of secondary compound, terpenoids.

DISCUSSION

In the screening program to the discovery and development of potential anticancer natural compounds, the criteria of the American National Cancer Institute was adopted, that a crude extract with IC_{50} lower than 30

Table 4. Absolute migration capability of the *Zingiberaceae* extracts on MDA-MB-231 cells measured using scratch wound assay.

Zingiberaceae species[1]	Solvent[2]	Concentration[3] (µg/ml)	Absolute migration capability, MC_A (mm/h)[4] MDA-MB-231 cell line[5], incubation status[6] 24 h	48 h
Control/untreated cells	-	0	7.35×10^{-3}	6.03×10^{-3}
Alpinia galanga	P	12.5	-8.75×10^{-4}	-5.73×10^{-4}
	C	12.5	-8.75×10^{-4}	-8.02×10^{-4}
	M	100	7.63×10^{-3}	5.78×10^{-3}
Boesenbergia rotunda	P	12.5	5.21×10^{-3}	2.98×10^{-3}
	C	12.5	4.29×10^{-3}	2.53×10^{-3}
	M	25	5.90×10^{-3}	4.54×10^{-3}
Curcuma aeruginosa	P	25	3.21×10^{-3}	1.93×10^{-3}
	C	50	1.31×10^{-3}	1.06×10^{-3}
	M	100	8.13×10^{-3}	7.04×10^{-3}
Curcuma domestica	P	12.5	4.96×10^{-3}	4.45×10^{-3}
	C	12.5	5.63×10^{-4}	1.35×10^{-4}
	M	12.5	3.27×10^{-3}	2.85×10^{-3}
Curcuma mangga	P	100	1.38×10^{-3}	1.17×10^{-3}
	C	25	1.90×10^{-3}	1.34×10^{-3}
	M	100	7.33×10^{-3}	5.72×10^{-3}
Curcuma xanthorrhiza	P	25	7.65×10^{-3}	5.83×10^{-3}
	C	12.5	4.94×10^{-3}	4.34×10^{-3}
	M	50	6.58×10^{-3}	5.42×10^{-3}
Kaempferia galanga	P	100	4.38×10^{-3}	2.71×10^{-3}
	C	25	4.73×10^{-3}	3.80×10^{-3}
	M	25	7.60×10^{-3}	5.68×10^{-3}
Zingiber montanum	P	25	3.65×10^{-3}	3.25×10^{-3}
	C	25	4.50×10^{-3}	3.90×10^{-3}
	M	100	6.08×10^{-3}	4.43×10^{-3}
Zingiber officinale	P	25	3.44×10^{-3}	2.69×10^{-3}
	C	25	5.96×10^{-3}	3.85×10^{-3}
	M	100	6.06×10^{-3}	4.81×10^{-3}
Zingiber zerumbet	P	12.5	2.63×10^{-3}	1.63×10^{-3}
	C	25	3.88×10^{-3}	2.54×10^{-3}
	M	100	5.65×10^{-3}	4.45×10^{-3}
[6]-Gingerol	-	12.5	5.38×10^{-3}	4.32×10^{-3}

[1]Ten selected *Zingiberaceae* rhizomes were selected for cell migration study using Scratch wound assay. [2] Solvents used in extraction: p: petroleum ether, c: chloroform, m: methanol; (-) not tested. [3] Crude *Zingiberaceae* extracts were tested at the classified concentration based on Table 3. [4] MC_A: Absolute migration capability (mm/h) calculated by Equation 2. [5] MDA-MB-231 (human breast cancer cell line, oestrogen-receptor negative). [6] Data was obtained at 24 and 48 h of incubation after treatment.

µg/ml was considered to be promising for further purification (Suffness and Pezzuto, 1990). Breast cancer is the second leading cause of cancer mortality affecting women in both developed and developing countries (Jemal et al., 2007). High incidence of mortality is also associated with poor prognosis for the metastatic disease, especially in hormone-independent cancer (Bange et al., 2001; Cuzick et al., 2004; Houssami et al., 2006; Roy et

Table 5. Qualitative analysis of crude chloroform extract of *Alpinia galanga* using thin-layer chromatography.

Labeled compound[1]	R_f[2]	Day light	UV light at 254 nm	UV light at 365 nm	Iodine vapour	50% H_2SO_4	Dragendorff's reagent	Vanillin/H_2SO_4 reagent	Types of compounds detected
A_1	0.05	-	-	-	brown	-	-	-	Compounds with double bonds
A_2	0.15	-	red	green	brown	-	-	purple	Compounds containing conjugated double bonds; fluorescent compounds; and terpenoids
A_3	0.18	-	-	-	brown	-	-	-	Compounds with double bonds
A_4	0.26	-	red	-	brown	-	-	-	Compounds with conjugated double bonds; and compounds with double bonds
A_5	0.35	-	red	-	-	-	-	-	Compounds containing conjugated double bonds
A_6	0.56	-	-	yellow	-	-	-	-	Fluorescent compounds
A_7	0.63	-	-	-	-	black	-	purple	Organic compounds; and terpenoids
A_8	0.65	-	red	green	brown	-	-	-	Compounds containing conjugated double bonds; fluorescent compounds; and compounds with double bonds.
A_9	0.71	-	red	-	-	-	-	-	Compounds containing conjugated double bonds
A_{10}	0.75	-	-	-	-	-	-	yellow	Phenols
A_{11}	0.79	-	red	-	brown	yellow	-	-	Compounds containing conjugated double bonds; compounds with double bonds; and organic compounds
A_{12}	0.83	-	red	-	brown	black	-	-	Compounds containing conjugated double bonds; compounds with double bonds; and organic compounds
A_{13}	0.85	Red	-	blue	brown	-	-	-	Coloured substances; fluorescent compounds; and compounds with double bonds
A_{14}	0.89	yellow	red	-	-	-	-	-	Coloured substances; compounds with conjugated double bonds
A_{15}	0.96	-	blue	-	brown	-	-	-	Compounds with conjugated double bonds; and compounds with double bond

[1]Resoluted compounds of crude chloroform extract of *Alpinia galanga* observed as bands on the stationary phase using several detection methods/spray reagents.[2]R_f: Retardation factor.

Table 6. Qualitative analysis of crude chloroform extract of *Curcuma domestica* using thin-layer chromatography.

Labeled compound[1]	R_f[2]	Day light	UV light at 254 nm	UV light at 365 nm	Iodine vapour	50% H_2SO_4	Dragendorff's reagent	Vanillin/H_2SO_4 reagent	Types of compounds detected
C_1	0.09	-	red	-	-	brown	orange	-	Compounds containing conjugated double bonds; organic compounds; and alkaloids
C_2	0.15	-	red	-	-	brown	-	-	Compounds containing conjugated double bonds; and organic compounds
C_3	0.25	-	red	-	-	brown	-	-	Compounds containing conjugated double bonds; and organic compounds
C_4	0.33	-	red	-	-	brown	-	yellow	Compounds containing conjugated double bonds; organic compounds; and phenols
C_5	0.49	-	red	-	-	black	-	purple	Compounds containing conjugated double bonds; organic compounds; and terpenoids
C_6	0.66	yellow	yellow	yellow	brown	black	orange	red	Coloured substances; compounds containing conjugated double bonds; fluorescent compounds; compounds with double bonds; organic compounds; alkaloids; and phenols
C_7	0.74	-	red	-	-	brown	-	-	Compounds containing conjugated double bonds; and organic compounds
C_8	0.80	-	red	-	-	brown	-	-	Compounds containing conjugated double bonds; and organic compounds
C_9	0.84	-	red	-	-	-	-	purple	Compounds containing conjugated double bonds; and terpenoids
C_{10}	0.88	-	red	-	-	-	-	-	Compounds containing conjugated double bonds
C_{11}	0.90	-		blue	-	-	-	-	Fluorescent compounds
C_{12}	0.95	-	red	-	brown	-	-	red	Compounds containing conjugated double bonds; compounds with double bonds; and phenols

[1]Resoluted compounds of crude chloroform extract of *Curcuma domestica* observed as bands on the stationary phase using several detection methods/spray reagents. [2]R_f: Retardation factor.

Table 7. Qualitative analysis of crude petroleum ether extract of *Zingiber zerumbet* using thin-layer chromatography.

Labeled compound[1]	R_f[2]	Daylight	UV light at 254 nm	UV light at 365 nm	Iodine vapour	50% H_2SO_4	Dragendorff's reagent	Vanillin/H_2SO_4 reagent	Types of compounds detected
Z_1	0.08	-	red	yellow	-	-	-	-	Compounds containing conjugated double bonds; and fluorescent compounds
Z_2	0.13	-	-	-	brown	brown	-	purple	Compounds with double bonds; oorganic compounds; and terpenoids
Z_3	0.16	yellow	-	-	-	-	-	purple	Coloured substances; and terpenoids
Z_4	0.20	-	red	yellow	-	-	-	-	Compounds containing conjugated double bonds; and fluorescent compounds
Z_5	0.23	-	-	blue	-	-	-	-	Fluorescent compounds
Z_6	0.38	-	-	-	brown	-	-	purple	Compounds with double bonds; and terpenoids
Z_7	0.59	-	-	-	-	brown	-	purple	Organic compounds; and terpenoids
Z_8	0.61	-	-	-	brown	brown	-	purple	Compounds with double bonds; organic compounds; and terpenoids
Z_9	0.63	-	red	-	-	-	-	-	Compounds containing conjugated double bonds
Z_{10}	0.64	yellow	-	-	brown	-	-	-	Coloured substances; and compounds with double bonds
Z_{11}	0.68	-	red	-	-	-	-	purple	Compounds containing conjugated double bonds; and terpenoids
Z_{12}	0.71	-	red	-	-	-	-	-	Compounds containing conjugated double bonds
Z_{13}	0.75	-	-	yellow	brown	-	-	-	Fluorescent compounds; and compounds with double bond
Z_{14}	0.90	-	-	blue	brown	-	-	Purple	Fluorescent compounds; compound with double bonds; and terpenoids
Z_{15}	0.98	-	-	yellow	-	-	-	-	Fluorescent compounds

[1]Resoluted compounds of crude petroleum ether extract of *Zingiber zerumbet* observed as bands on the stationary phase using several detection methods/spray reagents.[2]R_f: Retardation factor.

al., 2005). Based on this scenario, a highly metastatic oestroegen-independent human breast cancer cells, MDA-MB-231 was opted for this study. Metastasis is a complex and multi-steps process characterized by cell proliferation, cell adhesion, invasion, and migration (Chu et al., 2007; Lee et al., 2008). In the present study, anti-metastatic potentials of the Zingiberaceae extarcts were targeted on the proliferation and migration properties of MDA-MB-231 cells.

Anti-proliferation activity of the Zingiberaceae extracts were tested examined using MTT assay for 48 h. A significant dose-dependent anti-proliferative effect has been exhibited by 22 (73%) of the extracts, whereas eight other extracts (IC50 > 100 µg/ml) were inactive in inhibiting the proliferation or viability of MDA-MB-231 cells. Moreover, 12 (40%) of the active extracts possess IC_{50} values lower than 30 µg/ml. Based on Table 1, most of the active ones were petroleum ether and chloroform extracts, while methanol extracts were mostly showed no appreciation loss of the cell growth. The only methanol extract with IC_{50} < 30 µg/ml was methanol extract of *C. domestica*. These *Zingiberaceae* extracts possess chemopreventive properties as demonstrated by their ability to inhibit proliferations of MDA-MB-231 cells.

Anti-migration activities the *Zingiebraceae* extracts were examined on MDA-MB-231 cells using Scratch wound assay. The assay was used because the ability of tumour cells to migrate is closely associated to their metastatic potentiality. *In vitro* methods for cell migration study usually use system with 2D surfaces. *In vitro* 2D cell migration assays are prominently utilized because it is easy to be handled for screening tests that usually involve a large number of samples, which leads to the high statistical reliability of the results obtained (Entschladen et al., 2005). However, 2D model is inadequate since cell migration in organism or *in vivo* is a 3D system, which is more complicated. Nevertheless, 2D assays are very useful and appropriate for fundamental understanding of cell migration processes. Scratch wound assay involves both cell migration and proliferation (Hayot et al., 2006; Peng et al., 2006), 1% FBS DMEM was used as to exclude the possibilities that the observe effects of wound closure were due to cell proliferation.

The mechanism by which *Zingiberaceae* inhibits cell migration is not yet clear. However, it has been well documented that breast cancer cells secrete matrix metalloproteinases (MMPs), which promote cancer progression by boosting cancer cell proliferation, invasion, and migration (Kohn and Liotta, 1995; Lee et al., 2008). Involvement of multigene family of zinc-dependent endopeptidases of MMPs, particularly MMP-2 and MMP-9 in the aspect of cell-migration have been studied prominently (Kohn and Liotta, 1995). MMP-2 and MMP-9 play a crucial rule in proteolytic degradation of extra-cellular matrices (ECM) for regulating cancer cell migration process (Chena et al., 2005). In addition, overexpression of MMP-2 and MMP-9 stimulates angiogenesis, which speeds up cancer metastasis. Thus, new drug development by targeting this pathway is an important approach to control cancer metastasis. Cytotoxicity tests against cancer cell lines are the most common screening methods employed in the search for new anticancer drugs. Cytotoxicity assays currently applied in the developmental stages of anticancer drug are able to reveal compounds with the highest cyto-toxicity, but are not predictive of their potential toxicity. Toxicity studies are usually conducted on animals before clinical trials. The disadvantages of performing toxicity study right before clinical examination are time-consuming, expensive and require a large number of animals for the experiment (Popiolkiewicz et al., 2005). Moreover, there is always a high possibility that the substance causes high toxicity *in vivo*. Based on this scenario, it is vital to select the least toxic compounds among the active ones at the primary screening stage. A special parameter, known as selectivity index (SX) was applied in order to assess the selectivity of tested compounds towards tumour cells (Popiolkiewicz et al., 2005). In this context, it SX is defined as IC_{50} value obtained for MDA-MB-231 cells expressed as a percent of that of IC_{50} value from the test using MRC-5 cells. It acts as an objective indicator which enables evaluation of selectivity of the tested extracts between cancerous and non-cancerous cell lines. Thus, extract with SX value more than 100 were no doubt to hold a great promise for further studies. Six most cytotoxic extracts (IC_{50} ≤ 10 µg/ml) assessed in cell proliferation and viability assay were selected for *in vitro* toxicity testing against normal, non-tumoural MRC-5 cells.

The most active extract–petroleum extract of *A. galanga* with SX value 100 represented that its cyto-toxicity to kill both cancerous (MDA-MB-231) and non-cancerous (MRC-5) cells were similar, and thus can be excluded for further studies. Five other extracts possess SX values distinctly greater than 100, representing their promising values for further investigation in the development of anti-cancer drugs. This method enables appropriate estimation of the effective dose for toxicity tests *in vivo*, reduce the number of animals required for the tests, and more cost effective. It is a very efficient and applicable method for the selection of the most desirable substances.

Qualitative analysis using TLC was conducted for the three most promising extracts such as, chloroform extract of *A. galanga*, chloroform extract of *C. domestica*, and petroleum ether extract of *Z. zerumbet* for determining their nature substances. Most of the compounds detected from the chloroform extract of *A. galanga* (Table 5) contain conjugated double bonds and/or double bonds. Based on the previous studies, the main compounds found in the rhizome of *A. galanga* are phenylpropanoids, and most abundantly of which are 1'S-1'-acetoxychavicol acetate (Chappuis et al., 2007), 1'S-1'-acetoxyeugenol acetate (Laguna, 2003), and p-coumaryl diacetate

(Desjeux, 2001) etc. Most of the biological activities are due to the presence of phenylpropanoids (Kaur et al., 2010).

The compounds detected from the chloroform extract of *C. domestica* were mainly organic compounds with conjugated double bonds (Table 6). The rhizome of *C. domestica* consists of turmerin (a water-soluble peptide), essential oils (for example, turmerones, atlantones and zingiberene) and curcuminoids including curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (Sharma et al., 2005). Curcuminoids are phenolic compounds derived from turmerics, and curcumin commonly called diferuloylmethane is a hydrophobic polyphenol, which is generally regarded as the most active constituents of the rhizome of *C. domestica* (Anand et al., 2008). Curcumin has been a famous subject to be studied over the past three decades. It possesses antioxidant, anti-inflmmatory, cancer chemo-preventive and potentially chemotherapeutic properties (Anand et al., 2008; Sharma et al., 2005). In this study, anti-proliferation and anti-migration activities of the extracts may be correlated with the presence of curcumin. Based on the previous studies, volatile oils of the rhizomes contain zerumbone, humulene, and camphene (Nhareetsomchit and Nurshukriyah, 2003).

The major component of the rhizomes is zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-, (E,E,E)-), which is a monocyclic sesquiterpene containing cross-conjugated dienone system (Kitayama et al., 1999, 2002). Qualitative analysis of this extract using TLC indicated that 47% of the compounds detected were terpenoids. The anti-proliferation and anti-migration activities of the extracts may largely due to the presence of zerumbone.

To date, the present study is the first to demonstrate that varieties of local *Zingiberaceae* species can significantly inhibit metastatic process of MDA-MB-231 human breast cancer cells. Cancer metastasis is a complex process and therefore multi-assay strategies are needed to obtain maximum benefit from the screening of anti-migration drugs in order to identify the potential compounds. Further studies will be aimed at investigating the effects of chloroform extracts of *A. galanga*, chloroform extract of *C. domestica*, and petroleum ether extract of *Z. zerumbet* on the regulation of cellular mechanisms and upon isolating and identifying the substances responsible for their anti-metastatic effects.

ACKNOWLEDGEMENT

The research was financially supported by Postgraduate Research Fund (PPP) (PS 297-2010A) from University of Malaya, Malaysia.

REFERENCES

Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem. Pharmacol., 76: 1590-1611.

Bange J, Zwick E, Ullrich A (2001). Molecular targets for breast cancer therapy and prevention. Nat. Med., 7: 548-552.

Bode AM, Ma WY, Surh YJ, Dong Z (2001). Inhibition of epidermal growth factor-induced cell transformation and activator protein 1 activation by [6]-gingerol. Cancer Res., 61: 850-853.

Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, Alvar J, Boelaert M (2007). Visceral leishmaniasis: What are the needs for diagnosis treatment and control. Nat. Rev. Microbiol., 5: 873.

Chena PN, Hsieh YS, Chiou HL, Chub SC (2005). Silibinin inhibits cell invasion through inactivation of both P13K-Akt and MAPK signaling pathways. Chem. Biol. Interact., 156: 141-150.

Chu SC, Yang SF, Liu SJ, Kuo WH, Chang YZ, Hsieh YS (2007). In *vitro* and *in vivo* antimetastatic effects of *Terminalia catappa* L. leaves on lung cancer cells. Food Chem. Toxicol., 7: 1194-1201.

Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW (2004). Tamoxifen and breast density in woman at increased risk of breast cancer. J. Natl. Cancer Inst., 96: 621-628.

Desjeux P (2001). The increase in the risk factors for leishmaniasia worldwide. Trans. R. Soc. Trop. Med. Hyg., 95: 239.

Devasagayam TPA, Sainis KB (2002). Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J. Exp. Biol., 40: 639-655.

Entschladen F, Drell IV TL, Lang K, Mansur K, Palm D, Bastian P, Niggemann KS, Zaenker KS (2005). Analysis methods of human cell migration. Exp. Cell Res., 307: 418-426.

Fotsis T, Pepper M, Adlercreutz H, Hase T, Montesano R, Schweigerer L (1995). Genistein, a dietary ingested isoflavanoid, inhibits cell proliferation and *in vitro* anglogenests. J. Nutr., 125: 790-797S.

Hayot C, Debeir O, Van Ham P, Van Damme M, Kiss R, Decaestecker C (2006). Chartacterization of activities of actin-affecting drugs on tumor cell migration. Toxicol. Appl. Pharmacol., 211: 30-40.

Houghton PJ, Raman A (1998). Laboratory handbook for the fractionation of natural extracts. London: Chapman & Hall.

Houssami N, Cuzick J, Dixon JM (2006). The prevention, detection, and management of breast cancer. Med. J. Aust., 184: 230-234.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ (2007). Cancer statistics 2007. CA: Cancer J. Clin., 57: 43-66.

Kaur A, Singh R, Dey CS, Sharma SS, Bhutani KK, Singh IP (2010). Antileishmanial phenylpropanoids from *Alpinia galanga* (Linn.) Willd.. Indian J. Exp. Biol., 48: 314-317.

Kitayama T, Nagaoa R, Masuda T, Hill RK, Morita M, Takatani M, Sawada S, Okamoto T (2002). The chemistry of zerumbone IV: Asymmetric synthesis of zerumbol. J. Mol. Catal. B-Enzym., 17: 75-79.

Kitayama T, Okamoto T, Hill RK, Kawai Y, Takahashi S, Yonemori S, Yamamoto Y, Ohe K, Sawada S (1999). Chemistry of zerumbone. Simplified isolation, conjugate addition reactions, and a unique ring contracting transannular reaction of its dibromide. J. Org. Chem., 64: 2667-2672.

Kohn EC, Liotta LA (1995). Molecular insights into cancer invasion: Strategies for prevention and intervention. Cancer Res., 52: 1856-1862.

Laguna F (2003). Treatment of leishmaniasis in HIV-positive patients. Ann. Trop. Med. Parasitol., 97: S135.

Laundry de Mesquita M, Elias de Paula J, Pessoa C, Odorico de Moraes M, Costa-Lotufo LV, Grougnet R, Michel S, Tillequin F, Espindola LS (2009). Cytotoxic activity of Brazilian Cerrado plant used in traditional medicine against cancer cell lines. J. Ethnopharmacol., 123: 439-445.

Lee HS, Seo EY, Kang NE, Kim WK (2008). [6]-Gingerol inhibits metastasis of MDA-MB-231 human breast cancer cells. J. Nutr. Biochem., 19: 313-319.

Lin KL, Su JC, Chien CM, Tseng CH, Chen YL, Chang LS, Lin SR (2009). Naphthol[1,2-*b*]furan-4,5-dione induces apoptosis and S-phase arrest of MDA-MB-231 cells through JNK and ERK signaling activation. Toxicol. *In Vitro*, 17(19): 7021-7030.

Liu J, Zhang X, Yang F, Li T, Wei D, Ren Y (2005). Antimetastatic effect of a lipophilic ascorbic acid derivative with antioxidation through inhibition of tumor invasion. Cancer Chemother. Pharmacol., 2: 1-7.

Mossmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 16: 55-63.

Nhareetsomchit M, Nurshukriyah MH (2003). Anti-inflammatory property of ethanol and water extracts of Zingiber zerumbet. Indian J. Pharm., 35: 181-182.

Peng CC, Chen KC, Peng RY, Chyau CC, Su CH, Hsieh-Li HM (2007). Antrodia camphorate extract induces replicative senescence in superficial TCC, and inhibits the absolute migration capability in invasive bladder carcinoma cells. J. Ethnopharmacol., 109: 93-103.

Peng CC, Chen KC, Peng RY, Su CH, Hsieh-Li HM (2006). Human urinary bladder cancer T24 cells are susceptible to the Antrodia camphorata extracts. Cancer Lett., 243: 109-119.

Popiolkiewicz J, Polkowski K, Skierski JS, Mazurek AP (2005). *In vitro* toxicity evaluation in the development of new anti-cancer drugs-genistein glycosides. Cancer Lett., 229: 67-75.

Rogers AE, Zeisel SH, Groompman J (1993). Diet and carcinogenesis. Carcinogen., 14: 2205-2217.

Roy AM, Baliga MS, Katiyar SK (2005). Epigallocatechin-3-gallateinduces apoptosis in estrogen receptor-negative human breast carcinoma cells via modulation in protein expression of p53 and Bax and capase-3 activation. Mol. Cancer Ther., 4: 81-90.

Shanmugaraj S, Selvaraj S, Murugan VP, Rajaganapathy BR, Shanmuganathan MV, Devaraj S N, Subhadra LB (2010). 3β-Hydroxylup-20(29)-ene-27,28-dioic acid dimethyl ester, a novel natural product from *Plumbago zeylanica* inhibits the proliferation and migration of MDA-MB-231 cells. Chem. Biol Interact., 188: 412-420.

Sharma RA, Gescher AJ, Steward WP (2005). Curcumin: The story so far. Eur. J. Cancer, 41: 1955-1968.

Suffness M, Pezzuto JM (1990). Assays related to cancer drug discovery. In K. Hostettmann (ed) Methods in plant biochemistry: Assays for Bioactivity. London: Academic Press, pp. 71-133.

Surh Y (1999). Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. Mutat. Res., 428: 305-327.

Surh YJ (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. Food Chem. Toxicol., 40: 1091-1097.

Surh YJ (2003). Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer, 3: 768-780.

Surh YJ, Lee E, Lee JM (1998). Chemopreventive properties of some pungent ingredients present in red pepper and ginger. Mutat. Res., 402: 259-267.

Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M (2007). Growth inhibitory activity of cucurbitacin glucosides isolated from Citrullus colocynthis on human breast cancer cells. Biochem. Pharmacol., 73: 56-67.

Tsai SL, Suk FM, Wang CI, Liu DZ, Hou WC, Lin PJ, Hung LF, Liang YC (2007). Anti-tumor potential of 15,16-dihydrotanshinone I against breast adenocarcinoma through inducing G1 arrest and apoptosis. Biochem. Pharmacol., 74: 1575-1586.

Yap LC, Tang SW, Sukari MA, Ee CL, Rahmani M, Khalid K (2007). Characterization of flavonoid derivatives from *Boesenbergia rotunda* (L.). MJAS., 11: 154-159.