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## Anticancer Activity of Natural Compound (Zerumbone) Extracted from *Zingiber zerumbet* in Human HeLa Cervical Cancer Cells

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**Abstract:** A natural compound, zerumbone was extracted, isolated and purified from the rhizomes of edible plant *Zingiber zerumbet* using methanol extraction and Column Chromatography (CC) method. The isolated and purified zerumbone crystals were subjected to High Performance Liquid Chromatography (HPLC), Liquid Chromatography Mass Spectrometry (LCMS) and <sup>13</sup>C NMR and <sup>1</sup>H NMR analysis to confirm the purity, molecular weight and molecular structure. The study investigated the purified zerumbone crystals for its anti-cancer properties on human cervical cancer cell line (HeLa). Cisplatin, was used as a positive control in this study. The cytotoxicity of zerumbone and cisplatin were investigated using the MTT assay and caspases-3 was estimated with colorimetric assay in zerumbone treated HeLa cells. Morphological analysis showed that there were changes observed on HeLa cancer cells after treatment with zerumbone and cisplatin. The MTT assay results demonstrated that the IC<sub>50</sub> value ( $\pm$ SEM) of zerumbone was determined to be 11.3  $\mu$ M (2.5  $\mu$ g mL<sup>-1</sup>) whilst the IC<sub>50</sub> value of cisplatin was at 7.5  $\mu$ M (1.6  $\mu$ g mL<sup>-1</sup>). Prominent growth retardation was identified to the HeLa cancer cells, after treatment with both compounds, while caspase-3 was observed to be significantly increased in zerumbone treated cells as compared to untreated control cells. This study showed promising avenues towards zerumbone to be developed as a new chemo-natural drug for treatment of cervical cancer.

**Key words:** Zerumbone, cervical cancer, caspase-3, HPLC, LCMS, NMR

### INTRODUCTION

It has been estimated that there were 10.9 million new gynecological cancer cases globally, where 5.8 million were men while the women comprises 5.1 million cases. Worldwide, there were 6.7 million cancer deaths among, which 3.8 million were men and 2.9 million were women (Parkin *et al.*, 2005). Cervical cancer is the second most common cancer among women worldwide, with an estimated 493,000 new cases and 273,000 cancer deaths reported in 2002. The Malaysian National Registry reported that cervical cancer was the second most frequent neoplasm (12%) after breast cancer (30.4%) among Malaysian females in 2002. Thus initiatives are needed to circumvent the high number of reported cancer related cases as the number of cancer deaths are estimated to increase also. Since Peninsular Malaysia is covered mostly by tropical rainforest, efforts in search of

new cancer chemo-preventive compounds, in particular, those derived from medicinal herbal plants has its priorities set among local researchers. The forest which is yet unexplored has vast potential to discover newer generation of drugs useful for treatment of cancers. A noble concept of this cancer chemoprevention involves the use of natural pharmaceutical agents either to delay, inhibit or reverse the redevelopment of cancer before malignancy occurs (Chang *et al.*, 2000). This has been our main objective our present study for future treatment of all cancers.

World wide, considerable attention has been focused on herbal medicine which is based on the premise that these herbal plants may contain natural substances that can promote health and alleviate diseases. The World Health Organization estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the

use of plant extracts or their active components (Craig, 1999). Twenty five percent of modern drugs prescribed worldwide are plant-derived, where 121 of these active compounds are currently used in the treatment of various illnesses (Rates, 2001).

*Zingiber zerumbet* Smith, locally known as *lempoyang* wild ginger belongs to Zingiberaceae Family. It is native to South East Asia but has been widely cultivated plant in village gardens throughout the tropical and subtropical area around the world and has naturalized in some areas for its medicinal properties (Somchit and Shukriah, 2003). *Zingiber zerumbet* is used in local traditional medicine as a cure for swelling, sores and loss of appetite. Besides that the juice of the boiled rhizomes has also been used as a medicine for worm infestation in children. The volatile oils of the rhizomes have been shown to contain zerumbone, humulene and camphene. In some Southeast Asian countries, the rhizomes of the plant are employed as traditional medicines for anti-inflammation, while the young shoots and inflorescence are used as condiments (Murakami *et al.*, 2002).

The Zingiberaceae is one of Families in the order Zingiberales, which form an isolated group among the monocotyledons. About 1000 species out of 1400 species from 47 genera of Zingiberaceae were found in tropical Asia. There are about 25 genera with more than 160 species of Zingiberaceae in Peninsular, Malaysia. At least 20 or more ginger species have been cultivated for their use as spices, condiments, flavors, dyes, fresh vegetables, medicines, ornamentals and quite recently as cut flower (Habsah *et al.*, 2000). The rhizomes of Zingiberaceae family, namely *Curcuma domestica*, *Curcuma xanthorrhiza*, *Zingiber officinale* (red variety), *Zingiber cassumunar*, *Zingiber zerumbet* and *Kaempferia galangal* were found to express Epstein-Barr Virus early antigen (EBV-EA) activation inhibitory activity in Raji cells (Vimala *et al.*, 1999). Zerumbone, a monocyclic sesquiterpene from rhizomes of edible plant *Zingiber zerumbet* Smith was used locally as an anti-inflammatory medicine (D'Odorico *et al.*, 2001; Dev, 1960). Zerumbone found in some edible parts, including young stems and inflorescence, are used in traditional cooking (Kankuri *et al.*, 1999). The compound was shown to suppress tumour promoter 12-O-tetradecanoylphorbol-13 acetate (TPA)-induced Epstein-Barr virus activation in a potent manner (Murakami *et al.*, 2002). Zerumbone is known to be a potent suppressant of cyclooxygenase (COX)-2 and inducible nitric oxide synthases expression (Murakami *et al.*, 2003).

Nakamura *et al.* (2004) showed that zerumbone is one of the most promising chemopreventive agents against colon and skin cancer. Zerumbone was reported to suppress colonic tumour marker formation in rats and

induces apoptosis in colon cancer cell lines. The compound was shown to inhibit the proliferation of human colonic adenocarcinoma cell lines in a dose-dependent manner, while the growth of normal human dermal and colon fibroblast was less affected (Nakamura *et al.*, 2004). Zerumbone was further demonstrated to inhibit both azoxymethane-induced rat aberrant crypt foci and phorbol ester-induced papilloma formation in mouse skin a further indication of its efficacy to prevent colon and skin cancers (Tanaka *et al.*, 2001; Murakami *et al.*, 2004).

In present study, we have extracted, isolated, purified and identified the molecular weight and structure of a natural compound, zerumbone from the rhizomes of edible plant *Zingiber zerumbet*. The effects of zerumbone on cervical cancer cell line (HeLa) have not been investigated before. The objective of this study is to check the effects of this compound on the proliferation, cytotoxicity and apoptosis appearance in cervical cancer cells, HeLa cells.

## MATERIALS AND METHODS

Zerumbone was extracted from *Zingiber zerumbet* plant obtained from a wet market in Kuala Lumpur, Malaysia. All chemicals for extraction process were obtained from Sigma Aldrich. Chemicals, culture media and commercial drug Cisplatin were purchased from Sigma Aldrich. The cervical cancer cell lines (HeLa) was purchased from ATCC, USA. Cell culture plastic wares were from Nunc Company (Denmark). ApoTarget was a products of BioSource (USA). The MTT (Microtetrazolium) powder was purchased from Amresco and the DMSO (Dimethylsulphoxide) was purchased from Sigma Aldrich. The ELISA plate reader (Universal Microplate reader) was obtained from Biotech Instruments Inc, USA.

**Extraction and isolation of zerumbone from *Zingiber zerumbet* Smith.:** Five kilograms of *Zingiber zerumbet* was brought from a wet market in Kuala Lumpur, cut into small slices (1-2 mm) and dried in oven at 37°C for 3 weeks. The dried *Zingiber zerumbet* slices were ground and soaked in absolute methanol for 3 days and were filtered using filter paper. The methanol extract was collected in a conical flask (soaking was repeated 3 times). The methanol extract were rotor evaporated at 40°C using a rotor evaporator machine to obtain the crude extract. The crude extract was then mixed with methanol and distilled water and poured into separation funnel. Absolute hexane (100%) was added to the separation funnel and was shaken vigorously to mix well and left for 5 min for the solution to be separated into 2 layers. The compound zerumbone was obtained in the non-polar

layer. The aqueous and non-polar layers were separated in conical flask and the separation was repeated thrice. The non-polar layer from the separation process was poured into the round bottom flask to be rotor evaporated. The rotor evaporated extract from separation was added with silica gel and then rotor evaporated again until it forms a dried powder. Silica gel was mixed with hexane and poured into a glass the column, measuring 3.5×30.0 cm for chromatography.

**Column chromatography fractionation:** The powdered sample was poured into the silica gel column which later acts as an absorbent. The silica gel column was eluted with 100% hexane, followed by hexane and ethyl acetate mixture in a ratio of 9:1, 8.5:1.5 and finally 8:2, respectively. The ratio of 8:2 was maintained and repeated until the fraction containing zerumbone was isolated from the column. The fractions were collected in small vials (10 mL). The constituents in the fractions were determined using thin layer chromatography (TLC). The TLC of each fraction was compared with that of pure zerumbone compound. The fractions collected in vials were allowed to dry to form crystals. The zerumbone crystals undergo recrystallization to obtain purified zerumbone. These crystals were reconstituted and washed with hexane to remove all unwanted constituents thus allowing pure zerumbone crystals to recrystallize in the vials. The washing process was repeated 3 times. Pure zerumbone crystals were dried and later collected in clean vials.

**High performance liquid chromatography (HPLC) of isolated compound:** HPLC was performed using a JASCO-BORWIN Version 1.5 that consists of an auto sampler and quaternary solvent pump. The separation was done by using an analytical column GL Sciences Inersil C18 column (5  $\mu\text{m}$ , 2.1×150 mm). A mixture of 40% water and 60% Acetone nitrile was used as a mobile phase to separate the compound at flow rate of 0.2 mL min<sup>-1</sup>.

**Liquid chromatography mass spectrometry (LCMS) of isolated compound:** LCMS was performed using a Finnigan LC Q<sup>DECA</sup> (ThermoQuest) MS consisted of an auto sample and quaternary solvent pump. The separation was done by using an analytical column GL Sciences Inersil C18 column (5  $\mu\text{m}$ , 2.1×150 mm). A mixture of 40% 10 mM Ammonium acetate and 60% Acetone nitrile was used as a mobile phase to separate the compound at flow rate of 0.2 mL min<sup>-1</sup>. The LC system was connected to an ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (ACPI) interface in positive ion mode, a computer and a data acquisition/processing X-Caliber software system Version 1.2.

**Cell culture and maintenance:** Human cervical cancer cells (HeLa) was obtained from ATTC was grown in RPMI 1640 supplemented with 10% foetal calf serum, 1% penicillin-streptomycin and 1% amphotericin B. The flask containing HeLa cells were incubated in a humidified incubator with 5% CO<sub>2</sub>, at 37°C. Cultures were frequently examined under inverted microscope (Micros, Austria). Once the cells reached 80% confluency, media was removed and the cells were washed 3 times with 7 mL of PBS (Phosphate Buffer Saline). Two milliliters of trypsin was added to the cells and were incubated for 5 min. The flask was tapped gently to detach the cells from the wall of the flask to appear as single cells. Ten milliliters of RPMI 1640 with 10% FCS was added to the flask and the content of the flask was resuspended to allow the cells to disperse. About 6 mL of cell suspension was transferred into a 75 cm<sup>3</sup> flask. Ten milliliters of RPMI 1640 with 10% FCS was then added and incubated in CO<sub>2</sub> incubator at 37°C. The cells were frequently examined under an inverted microscope for confluency and viability.

**Cell survival/cytotoxicity assay:** One milligram of zerumbone and cisplatin were measured and diluted in 100  $\mu\text{L}$  of 100% ethanol, respectively. Nine hundred microliters of RPMI 1640 was added to prepare stock solution at the concentration of 1 mg mL<sup>-1</sup>. Sub stock solution of 100  $\mu\text{M}$  zerumbone and 100  $\mu\text{M}$  cisplatin were prepared from the stock solution 1 mg mL<sup>-1</sup>. HeLa cells were washed 3 times with 7 mL of PBS. Two and half milliliters of trypsin were added to the cells and were incubated for 5 min in the CO<sub>2</sub> incubator. Once the cells were detached from the flask, 10 mL of RPMI with 5% FCS was added into the flask. Cells density was determined using a hemocytometer. One hundred microliters of cell suspension were plated in each well of 96 well plates at concentration of 1×10<sup>5</sup> cells mL<sup>-1</sup>. After 24 h incubation, content of each well was decanted and cells were treated with different concentrations of zerumbone and cisplatin. The cells were incubated in CO<sub>2</sub> incubator at 37°C for 3 days (72 h). Twenty microliters of 5 mg mL<sup>-1</sup> MTT (Micro culture Tetrazolium) solution was added into to each well. The plate was covered with aluminum foil and incubated at 37°C (5% CO<sub>2</sub>) for 4 h in dark in order to allow the active live cells to convert water soluble yellow MTT solution into water insoluble purple formazan. After 4 h of incubation, the media containing MTT solution was aspirated. The remaining purple formazan was dissolved by adding 100  $\mu\text{L}$  DMSO into each well. The absorbance of the formazan was measured at 450 nm wavelength using ELISA plate reader. The IC<sub>50</sub> value (concentration at

which 50% of the cells are viable and another 50% cells killed) was determined from the dose-response curve (% cell viability versus concentration of zerumbone or cisplatin).

**Morphological studies:** The cells were washed 3 times with 7 mL of PBS and 2.5 mL of trypsin was added to the cells and were incubated for 5 min in the CO<sub>2</sub> incubator. Once the cells were detached from the flask, 10 mL of RPMI with 5% FCS was added. The cells viability was determine using hemocytometer and 2 mL of cells were plated in each well of 12 wells plate at concentrations of  $1 \times 10^5$  cells mL<sup>-1</sup>. Next day, the content of each well was decanted and was treated with zerumbone and cisplatin at their respective IC<sub>50</sub> values (IC<sub>50</sub> value for zerumbone was 10.8 μM and for cisplatin was 5.3 μM). Two milliliters of media were added into control wells without treatment. The cells were incubated at 37°C in 5% CO<sub>2</sub> incubator. The morphological features of the cells were examined under an inverted microscope at 24, 48 and 72 h after treatment.

**Caspase-3 activity assay:** After treatment by the compounds, the caspase-3 activity of the cell lysate was determined using a caspase colorimetric assay kit, according to the supplier's manual. The optical density of the assay solutions was measured using a spectrophotometer at 405 nm.

**Data analysis:** The data are presented as mean±SEM and the statistical significance between treatment and control group was analyzed by Student t-test. The differences between groups were regarded as significant at  $p < 0.05$ .

## RESULTS

**High performance liquid chromatography analysis:** HPLC analysis of the sample solution containing the said compound, zerumbone showed only one single high peak with a retention time of 16 min (Fig. 1). This single peak ensures purity of the compound isolated, which exemplified further that the compound extracted and isolated from crude plant extract consisted mainly as one single compound. Though a single peak was obtained following HPLC analysis, further analysis using LCMS was needed in order to identify the molecular weight of the compound isolated. This is crucial to further substantiate that the compound identified was indeed the intended compound investigated.

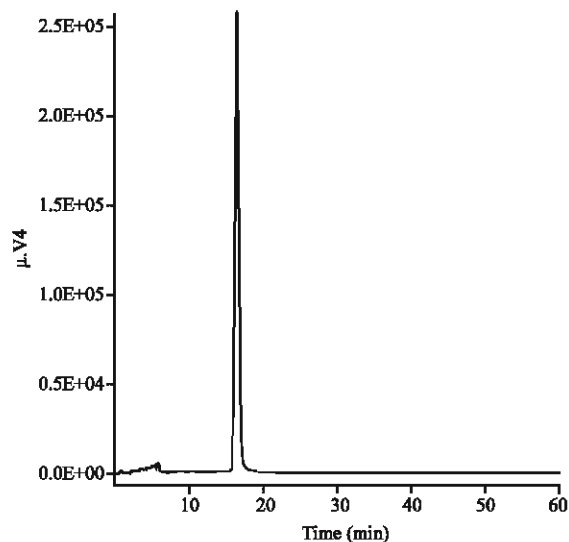


Fig. 1: HPLC analysis graph of the isolated compound showed only one single peak at retention time of 16 min. No other peaks were found and this demonstrated purity of the compound extracted and isolated

**Liquid chromatography mass spectrum analysis of extracted sample:** The mass spectra of zerumbone were studied in positive ionization mode. The most intense ions in the full mass spectrum are the protonated (M+H)<sup>+</sup> molecules. LCMS analysis showed that the compound had generated a high fragment with molecular ions at  $m/z$  219 (M+1)<sup>+</sup> under positive scan modes. The molecular weight of this fragment can be derived from the positive ions (positive ions:  $219 - 1 = 218$ ). Based on this calculation, the molecular weight of the fragment is 218, which is the molecular weight of the compound, zerumbone. This fragment is the most abundant in Mass Spectrometry graph (Fig. 2). The compound also generated a fragment ion under positive product ion scan mode (MS/MS) at  $m/z$  259, which can be assigned as the product ion due to a loss of a molecule from the precursor ion. In the following analysis conducted, only product ions with relative abundance greater than 25% in the spectra were considered. Since the fragment at  $m/z$  259 has relative abundance less than 25%, it is not considered. This finding duly confirms that the compound isolated is zerumbone.

**<sup>13</sup>C NMR and <sup>1</sup>H NMR analysis of extracted sample:** Zerumbone was isolated as pale yellow crystals. The LCMS spectrum was showed the presence of a molecular ion peak at  $m/z$  218 which corresponds to a molecular

Position	δ <sup>1</sup> H (500 MHz, CD <sub>3</sub> OD)	δ <sup>13</sup> C (125 MHz, CD <sub>3</sub> OD)
1	2.243-2.293 (m, 2H)	42.142
2	1.900-1.925 (d, 1H)	124.905
3		136.457
4	2.367-2.445 (m, 2H)	39.210
5	2.498-2.544 (m, 2H)	24.261
6	6.083-6.106 (d, 1H)	149.971
7		137.747
8		205.538
9	5.326-5.295 (d, 1H)	126.932
10	5.968 (s, 1H)	161.942
11		37.683
12	1.573 (s, 3H)	14.228
13	1.770 (s, 3H)	10.724
14	1.086 (s, 3H)	23.360
15	1.238 (s, 3H)	28.659

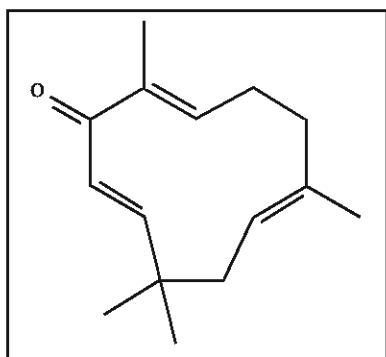


Fig. 2: Molecular structure of zerumbone

formula C<sub>15</sub>H<sub>22</sub>O. The <sup>1</sup>H-NMR spectrum showed four singlets which resonates at δ 1.573, 1.770, 1.086 and 1.238 were due to the methyl group at C-12, C-13, C-14 and C-15. A broad duplet was found between δ 6.083- 6.106 at C-6 position. Three multiplets between δ 2.243-2.544 were attributed to the methylene groups at position C-1, C-4 and C-5. The C-NMR spectrum gave a total of fifteen carbons. The most downfield signal at δ 205.528 was due to the carbonyl group at position C-8. The signal of methyl group at position C-12, C-13, C-14 and C-15 were observed at δ 14.228, 10.724, 23.360 and 28.659 (Table 1). This analysis coincides to the chemical structure that resembles zerumbone, as shown in Fig. 2.

**Cell survival/cytotoxicity assay:** Micro-Tetrazolium (MTT) cytotoxicity assay was performed on human cervical cancer cells, HeLa after treatment with compounds, zerumbone and cisplatin. Figure 3 and 4 illustrate the cytotoxicity effects of zerumbone and cisplatin on HeLa cancer cells respectively. The graph showed that the IC<sub>50</sub> value (±SEM) of zerumbone was determined to be 11.3 μM (2.5 μg mL<sup>-1</sup>) whilst the IC<sub>50</sub> value of cisplatin was 7.5 μM (1.6 μg mL<sup>-1</sup>). The results showed that cisplatin exerted better cytotoxic effects on HeLa cancer cells at lower concentration (7.5 μM) as

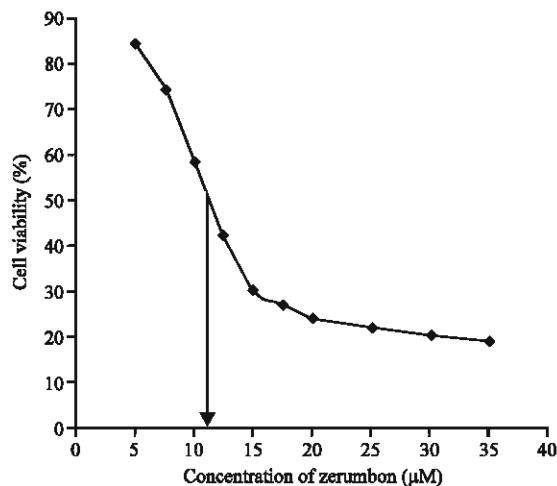


Fig. 3: The effect of zerumbone on, HeLa cells after 72 h post-treatment at concentrations, 5 to 35 μM. The IC<sub>50</sub> value of zerumbone was determined to be 11.3 μM±0.2 (±SEM) (p<0.01)

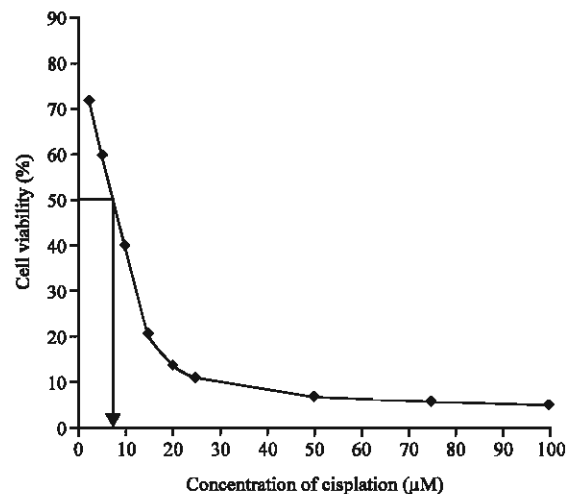


Fig. 4: The effect of cisplatin on HeLa cells after 72 h of post-treatment at concentrations, 2.5-100 μM. The IC<sub>50</sub> value of cisplatin was determined at 7.5 μM±0.3 (±SEM) (p<0.01)

compared to zerumbone, which needed higher concentration (11.3 μM) to induce similar effects. There is however, a significant difference in the IC<sub>50</sub> value of both zerumbone and cisplatin on HeLa cancer cells with (p<0.01) as analyzed using Independent sample t-test. Even though cisplatin have lower IC<sub>50</sub> value compared to zerumbone, nonetheless, both IC<sub>50</sub> values of zerumbone and cisplatin falls within the very significant range (= 4 μg mL<sup>-1</sup>) of cytotoxic effects, as established by the National Cancer Institute Standard, United States, 1972. 25.

**Morphological studies using inverted microscopy:** There was significant morphological changes observed to the HeLa cancer cells during treatment with zerumbone and cisplatin, after 24, 48 and 72 h post-addition. Prominent growth retardation was identified to the HeLa cancer cells, after treatment with zerumbone and cisplatin, as compared to untreated control cells, the later observed to have rapid cell growth. At 24 h post-treatment, few rounded cancer cells (cell shrinkage) were observed after zerumbone treatment, whilst the cancer cells treated with cisplatin showed more rounded cancer cells with budding cell

membrane (Fig. 5). An increased number of rounded cancer cells were observed at 48 h post-treatment with zerumbone. However at similar time interval, HeLa cancer cells treated with cisplatin showed more abundant rounded cells as compared relatively to zerumbone (Fig. 6). A progressive nuclear shrinkage with increased rounded cancer cells was noticeable to the HeLa cells treated with zerumbone at 72 h post-treatment as compared to the 72 h post-treatment with cisplatin which resulted in higher increased of rounded cells (Fig. 7).

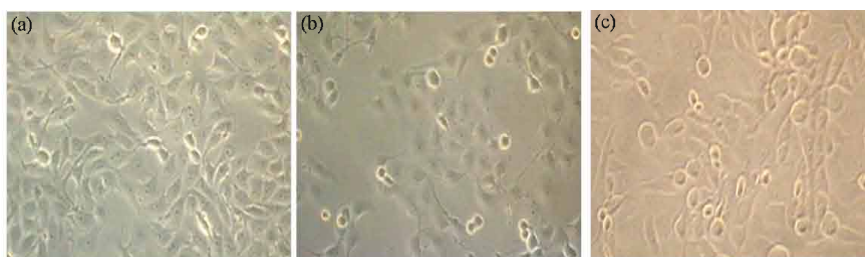


Fig. 5: Micrographs showing morphological changes towards HeLa cells as viewed under light contrasting inverted microscope after 24 h post-treatment with zerumbone and cisplatin. (a) untreated control cancer cells, viewed to have normal rapid growth (b) treatment with zerumbone and (c) cisplatin, both compounds exhibiting effects of cell growth inhibition and noticeable rounded cells as compared to control (Magnification, 20x10)

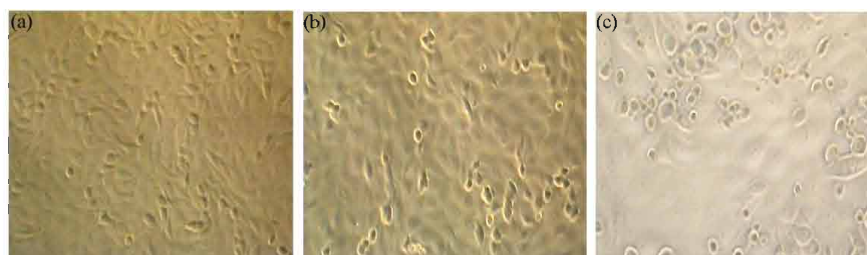


Fig. 6: Micrographs showing morphological changes towards HeLa cells as viewed under light contrasting inverted microscope after 48 h post-treatment with compounds, zerumbone and cisplatin. (a) untreated control cells of confluent cancer cells (b) treatment of HeLa with zerumbone and (c) cisplatin, both demonstrating respective effects towards the cancer cells of reduced cell growth proliferation and rounded cells compared with untreated control cells (Magnification 20x10)

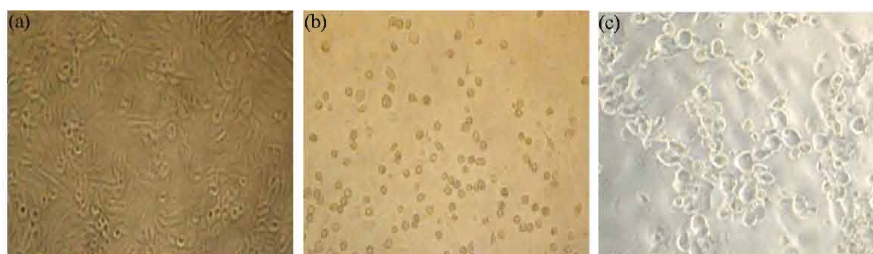


Fig. 7: Micrographs showing morphological changes towards HeLa cells as viewed under light contrasting inverted microscope after 72 h post-treatment with compounds, zerumbone and cisplatin. (a) untreated control of confluent cancer cells (b) treatment of HeLa with zerumbone and (c) cisplatin, both demonstrating effects towards the cancer cells of reduced cell proliferation and rounded cells, with added features of nuclear shrinkage following zerumbone treatment as compared to untreated control cells (Magnification 20x10)

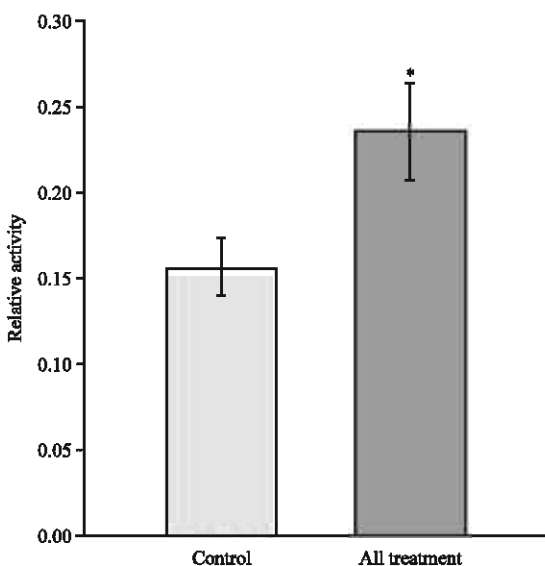


Fig. 8: The effects of zerumbone on caspase-3 activity in human cervical cancer cells (HeLa cell line) compared to untreated control cells. \* $p < 0.05$

**The effect of zerumbone on caspase-3 activity:** Caspase-3 is a major mediator of apoptosis acting downstream of the mitochondrial signaling pathway. As shown in Fig. 8, after exposure to the zerumbone, the activity of caspase-3 significantly increased compared to the untreated control cells.

## DISCUSSION

Since cervical cancer is the second most common cancer among women worldwide, it continues to be a serious health problem. Cisplatin is often used in combination with 5-fluorouracil and considered as the gold standard treatment for women with locally advanced cancer of the cervix. These chemotherapeutic drugs destroy cancer cells by interfering the cells division and growth. The affected cells become damaged and eventually die. However, apart from affecting the cancer cells, these chemotherapeutic drugs also affect normal cells. The use of natural compounds extracted from fruits, vegetables, oil seeds and herbs as an antioxidant and functional foods has become a global trend recently (Wang *et al.*, 1997). Zerumbone, a sesquiterpenoid compound, isolated from the methanol extract of rhizome from edible plant, *Zingiber zerumbet* Smith, has  $\alpha$ ,  $\beta$ -unsaturated carbonyl group at the 8-position which plays an important role in the inhibition of tumor cell growth (Murakami *et al.*, 2002; Matthes *et al.*, 1980).

The HPLC analysis of the compound extracted showed one single high peak at retention time of 16 min. This single peak demonstrated the purity of the

compound isolated. To identify that the compound isolated is zerumbone, further analysis using Liquid Chromatography Mass Spectrometry (LCMS) was done. The Chromatogram of LCMS showed a single high fragment with a relative abundance of 100%. The molecular weight of this fragment was identified as 218, which has similar molecular weight to zerumbone. This finding confirms that the isolated compound achieved purity of 99.99%, which was identified as zerumbone. HPLC and LCMS analysis proves that the methanol mixture obtained following the modification of extraction and isolation method had successfully isolated a purified compound identified to that of zerumbone. The extraction method that incorporated both adapted an organic extraction and chromatographic chemistry is capable of isolating zerumbone from other interfering and unknown chemical compounds. This further entails that the method employed has successfully extracted, isolated and purified the intended compound at 99.99% purity.

Cisplatin, a commercial drug used to treat cervical cancer was compared with zerumbone on HeLa cells. The present MTT assay result demonstrated that zerumbone effectively inhibit cell proliferation of human cervical cancer cells (HeLa) in a dose-dependent manner. Both the  $IC_{50}$  values of cisplatin and zerumbone falls in the very significant category based on the NCI standard. Significant morphological changes were also observed in HeLa cancer cells following treatment with zerumbone and cisplatin after 24, 48 and 72 h post-treatment. Prominent growth retardation and shrunken (rounded) cells were noticed at 72 h post-treatment with both zerumbone and cisplatin whilst control untreated HeLa cancer cells were well spread and confluent at 72 h. This indicates that both zerumbone and cisplatin are able to induce cell death effectively. The MTT assay and morphological analysis of this study showed that both cisplatin and zerumbone induce cell death in HeLa cancer cells. The findings of this study showed that zerumbone acts similarly to cisplatin. Caspases are cysteine proteases that play fundamental roles in the apoptotic responses of cells to different stimuli. Since caspases such as caspase-3 is a main executioner of the apoptotic response inside of the cell (Nunez *et al.*, 1998). The activity of caspase-3 in zerumbone treated cervical cancer cells as examined and the results of caspase-3 activity was confirmed that zerumbone action is by inducing apoptosis.

Apart from this recent study conducted, zerumbone was also shown to exhibit cytotoxic activities in other tissues. Zerumbone has been shown to exhibit cytotoxic activities on Hepatoma Tissue Culture (HTC), a neoplastic rat liver cell strain cultured *in vitro* and was found to be selective against normal mouse fibroblast (Murakami *et al.*, 1980). Murakami *et al.* (2002) showed



that zerumbone inhibit the proliferation of human colonic adenocarcinoma cell lines (LS 174T, LS 180, COLO 205 and COLO 320DM) in dose-dependent manner, while the growth of normal human dermal (2FO-C25) and colon fibroblasts (CCD-18 Co) was less affected. In another study (Tanaka *et al.*, 2001) zerumbone has been implicated as one of the promising chemopreventive agents against colon and skin cancer. The  $\alpha,\beta$ -unsaturated carbonyl group at the 8-position in zerumbone is thought to play an important role as an anti-tumour agent. As stated by Murakami *et al.* (2002) humulene, a structural analogue of zerumbone lacking only the carbonyl group in zerumbone was virtually inactive in all experiments involving tumour cells. This was later confirmed by Murakami *et al.* (2004), who further reported similar findings that the carbonyl group at the 8-position in zerumbone is the important structural element for its chemopreventive potential.

Naturally occurring substances that block or suppress the proliferation of tumour cells are potentially potent antitumor agents. Anticancer agents from edible plants have an added advantage in their clinical application on account of their low toxicities (Vimala *et al.*, 1999). This study suggests that zerumbone is effective as an anticancer agent and have a chemotherapeutic potential on cervical cancer cells. Cisplatin is often used in combination with one, two, three or even four other drugs with good results. Studies concluded that the combination of both cisplatin and taxol induce apoptosis in epithelial ovarian cancer (Havrilesky *et al.*, 1995; Ormerod *et al.*, 1996). Hence, this study may also suggest the possibility of using zerumbone as a new combination drug with cisplatin in treating cervical cancer.

### CONCLUSION

In the present study, the effectiveness of zerumbone as an anti-cancer agent was compared to a commercial drug, cisplatin used preferentially to treat cervical cancer. *In vitro* studies of zerumbone and cisplatin towards HeLa cancer cells showed that both compounds are able to induce cell death towards the cancer cells in possibly, similar mechanistic action. Therefore, this study showed promising avenues to the compound zerumbone, as a new chemo-natural drug for future treatment of cervical cancer.

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