

## **Anti-proliferative effect of Thai watermelon leaf extracts on cervical and breast cancer cells**

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### **Abstract**

Cervical and breast cancers are the most two common cancers and leading cause of women death. Major problems of treating these cancers with the existing chemotherapeutic drugs are the adverse side effect and drug resistance. Hence, in this study, the anti-proliferative effects of leaf extracts from six cultivars of Thai watermelon (*Citrullus lanatus*) were evaluated against cervical carcinoma C33A, HeLa and SiHa cells as well as breast adenocarcinoma MDA-MB-231 and MCF-7 cells, in comparison to non-cancerous Vero cell. MTT results demonstrated the reduced viability of cancer cells exposed to leaf extracts, especially those from Zonya and King orange cultivars. Cancer cells showed smaller size and cell number was reduced while no such negative effect was observed in Vero cells. These results indicate the specificity of extracts against cancer over normal cells and possibility to develop these watermelon leaf extracts as the novel anti-cancer agents.

*Keywords: Watermelon leaf extract, anti-proliferative activity, breast cancer cells, cervical cancer cells*

### **Introduction**

Breast and cervical cancers are the two leading cause of death in women worldwide including Thailand and also are the two highest estimated new cancer cases in the developing countries (*Public Health Statistics*, 2015; Torre et al., 2015). Among many types of cancer treatment, chemotherapy is the most common method. Major problems of treating breast and cervical cancers with the existing chemotherapeutic drugs are the adverse side effect and drug resistance of cancer cells. Natural anti-cancer compounds might help to solve these problems. Extracts and isolated compounds from several medicinal plants have been previously demonstrated for their cytotoxicity on breast and cervical cancer cells (Rahman et al., 2011; Siriwatanametanon et al., 2010).

Watermelon (*Citrullus lanatus*, Cucurbitaceae family) is a popular fruit in tropical and Mediterranean countries. Watermelon flesh is a very rich source of lycopene, beta-carotene and various vitamins including A and C, which exert the antioxidant and also anti-tumor properties (Adetutu et al., 2015). Extracts from seed and peel also possessed antioxidant capacity (Asghar et al., 2012; Tabiri et al., 2016). However, leaf of watermelon is still a wasteful product. Previously, the leaf extract exhibited the highest antioxidant and anti-inflammation activities among other parts (Thongtha, 2016); therefore, it was thus selected to evaluate anti-proliferative potential against breast and cervical cancer cell lines in this study.

## Materials and Methods

### Plant materials

The leaves of six cultivars of *C. lanatus* including Zonya (ZY), Yaya (YY), Kinnaree (KN), Diana (DN), Apollo (AO) and King Orange (KO) were obtained from Nuch-cha Thai Melon Co.,Ltd, Ban Bueng District, Chonburi, in July 2016.

### Extract preparation

Leaves were cleaned with tap water and dried in a hot-air oven at 50 °C. The fine powder of dried leaves was soaked in 95% ethanol (1 g: 10 mL) for 5 days (3 times) with occasionally shaking. After that, all filtrates were evaporated using rotary evaporator and freeze dryer.

### Cell culture condition

Human cervical carcinoma cell lines (C33A, HeLa and SiHa), human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231) and non-cancerous cell line (Vero) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (10<sup>4</sup> µg/mL/10 mg/mL), and incubated in an incubator with 5% CO<sub>2</sub> at 37 °C.

### Cell viability assay

The effect of watermelon leaf extract on viability of cell lines were measured by the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as previously described (Wanichwatanadecha et al., 2012). Cells were trypsinized and seeded into 96-well plate at a density of 2.5x10<sup>3</sup> cells per well (for SiHa, HeLa, MDA-MB-231 and Vero) or 5x10<sup>3</sup> cells per well (for C33A and MCF-7). After overnight incubation, cells were treated with different concentrations (0-400 µg/ml) of extracts and incubated for 0, 24, 48, or 72 hours. 0.2%DMSO (v/v) was used as a negative control. After that, the medium was changed into MTT-containing medium (0.5 mg/ml) and incubated for additional 3 hours. Then the medium was removed and 200 µl of DMSO was added to dissolve the reduced formazan crystals. Finally, the absorbance (A) of the formazan product was measured at a wavelength of 540 nm using a microplate reader (VersaMax, USA). The cell viability was calculated as equation (1) and concentration of extract that could inhibit cell proliferation by 50% was also expressed as half maximal inhibitory concentration (IC<sub>50</sub>). The experiment was done in triplicate with three independent experiments and expressed as mean± SD.

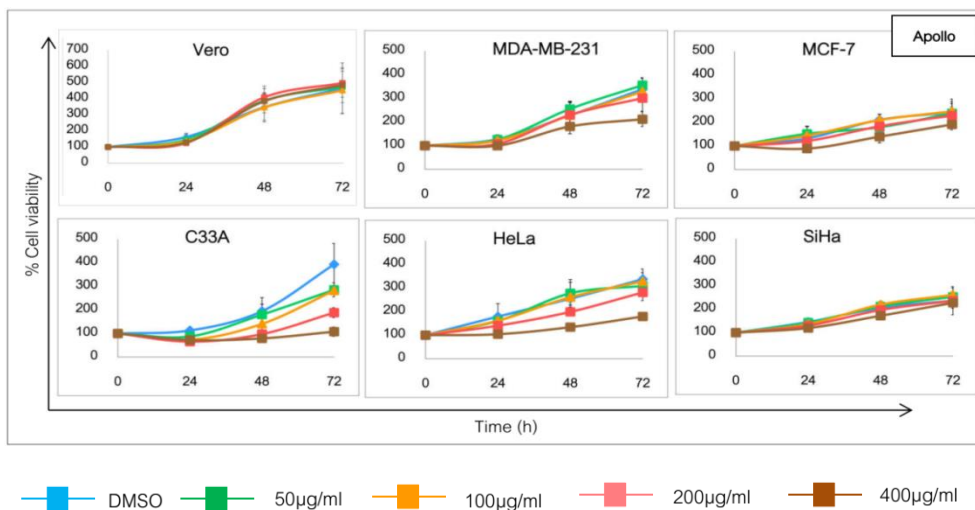
$$\% \text{ cell viability} = \frac{[A_{540}(\text{treated}) - A_{540}(\text{blank})]}{[A_{540}(\text{untreated}) - A_{540}(\text{blank})]} \times 100 \quad (1)$$

### Cell morphology and cell number observation

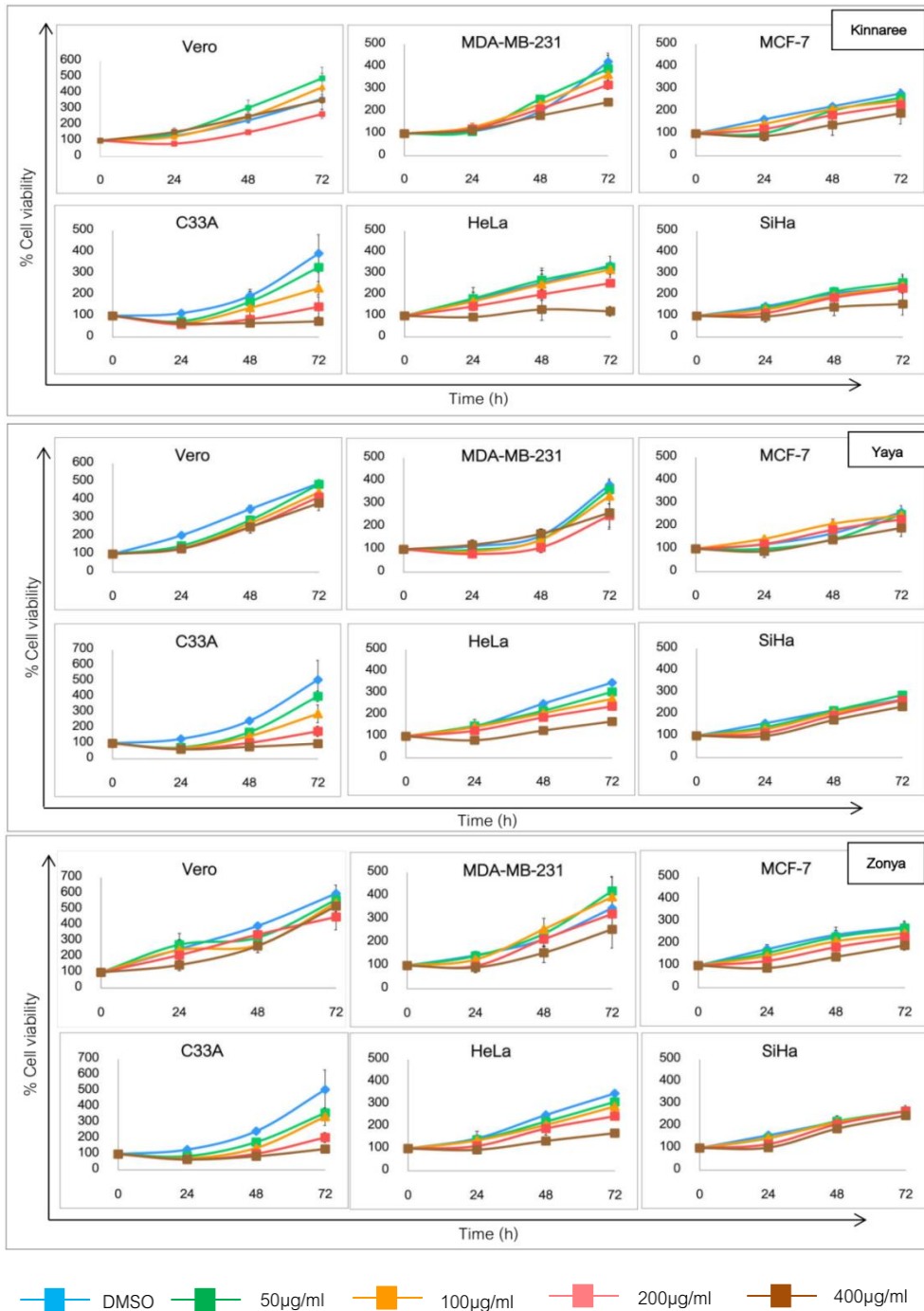
Effects of extracts on cell morphology and cell number were observed under inverted microscope (Nikon Eclipse TS100, Japan). Selected cells were exposed to 0.2% (v/v) DMSO or extracts (ZY or KO) at IC<sub>50</sub> for 72 hours. The photographs were taken directly from culture plates under inverted microscope at 10X objective lens magnification.

## Results

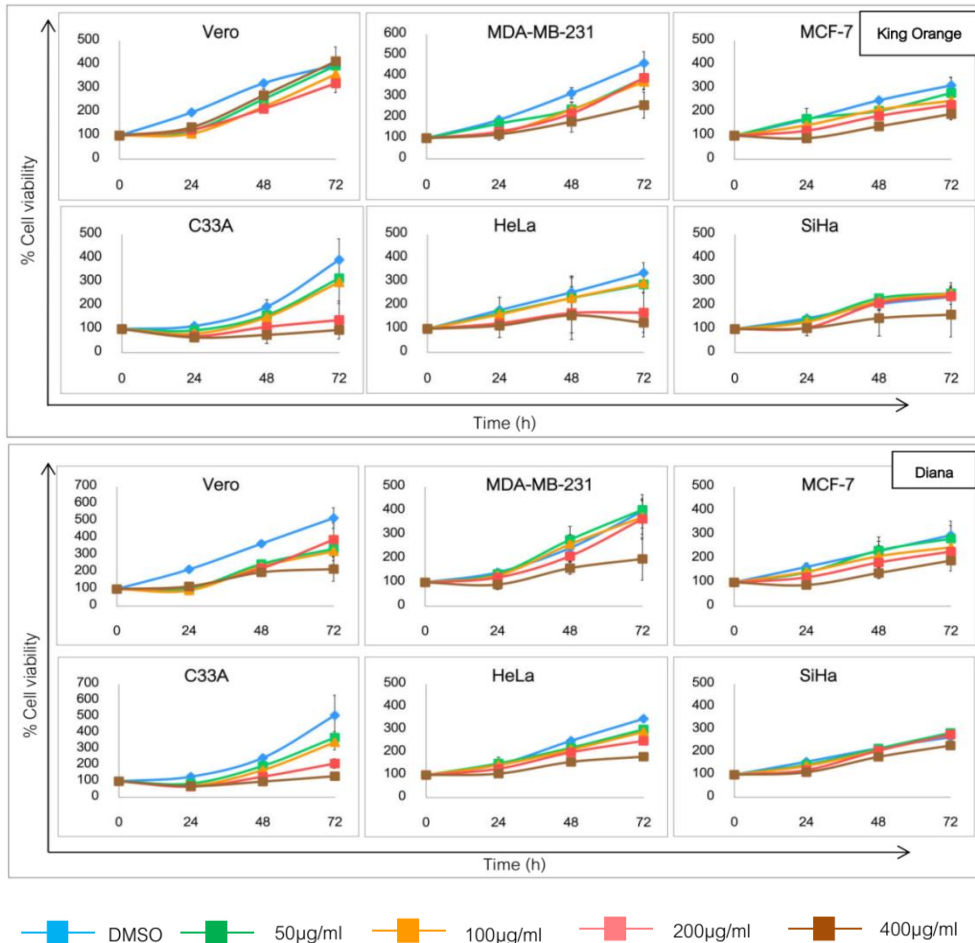
The effects of watermelon (*C. lanatus*) leaf extracts on viability of cervical and breast cancer cells as well as non-cancerous cells were evaluated by MTT assay (Figure 1). The results demonstrated that all leaf extracts from six cultivars of Thai *C. lanatus* had anti-proliferative activity against all cancer cells with different degrees. Plants in Cucurbitaceae family as *C. lanatus* have been previously reported their anti-cancer activity from leaf extracts and isolated compounds against various types of cancer (Jayaprakasam et al., 2003; Raina et al., 2016). MTT results also showed that the viability of cervical cancer cells was lower than that of breast cancer cells when exposed to the leaf extracts. In addition, it was shown to depend on extract concentration and incubation time. Among different cell lines, C33A was the most sensitive cancer cells with a clear reduction in growth when exposed to any extracts ( $IC_{50} = 127-177 \mu\text{g/mL}$ , Table 1). Among six extracts, Kinnaree (KN), Yaya (YY) and King orange (KO) exhibited the high anti-cervical cancer activity, however, KN was also toxic to Vero cells while no such effect was observed from KO and YY. In comparison between KO and YY, both extracts revealed the similar cytotoxicity toward two out of three cervical cancer cells (C33A and HeLa) with comparable  $IC_{50}$  against C33A cells, but KO had the much lower  $IC_{50}$  against HeLa cells than YY (185 and 378  $\mu\text{g/mL}$ , respectively). Zonya (ZY) and Diana (DN) extracts showed the broad-range inhibitory effect on both breast cancer cell lines, but only ZY showed no negative effect on Vero cells. The higher sensitivity of MDA-MB-231 than MCF-7 cells to the extracts was similar to the previous study treating cells with isolated compound (Cucurbitacin B) from plant in the same family as *C. lanatus* (*Trichosanthes cucumerina* L.) (Duangmano et al., 2012).



**Figure 1** Cell viability of cancer and non-cancerous cells after treatment with various concentrations of leaf extracts from six cultivars of watermelon for 0, 24, 48, or 72 hours. %Cell viability at 0-h incubation of each concentration was set as 100%.



**Figure 1 (cont.)** Cell viability of cancer and non-cancerous cells after treatment with various concentrations of leaf extracts from six cultivars of watermelon for 0, 24, 48, or 72 hours. %Cell viability at 0-h incubation of each concentration was set as 100%.

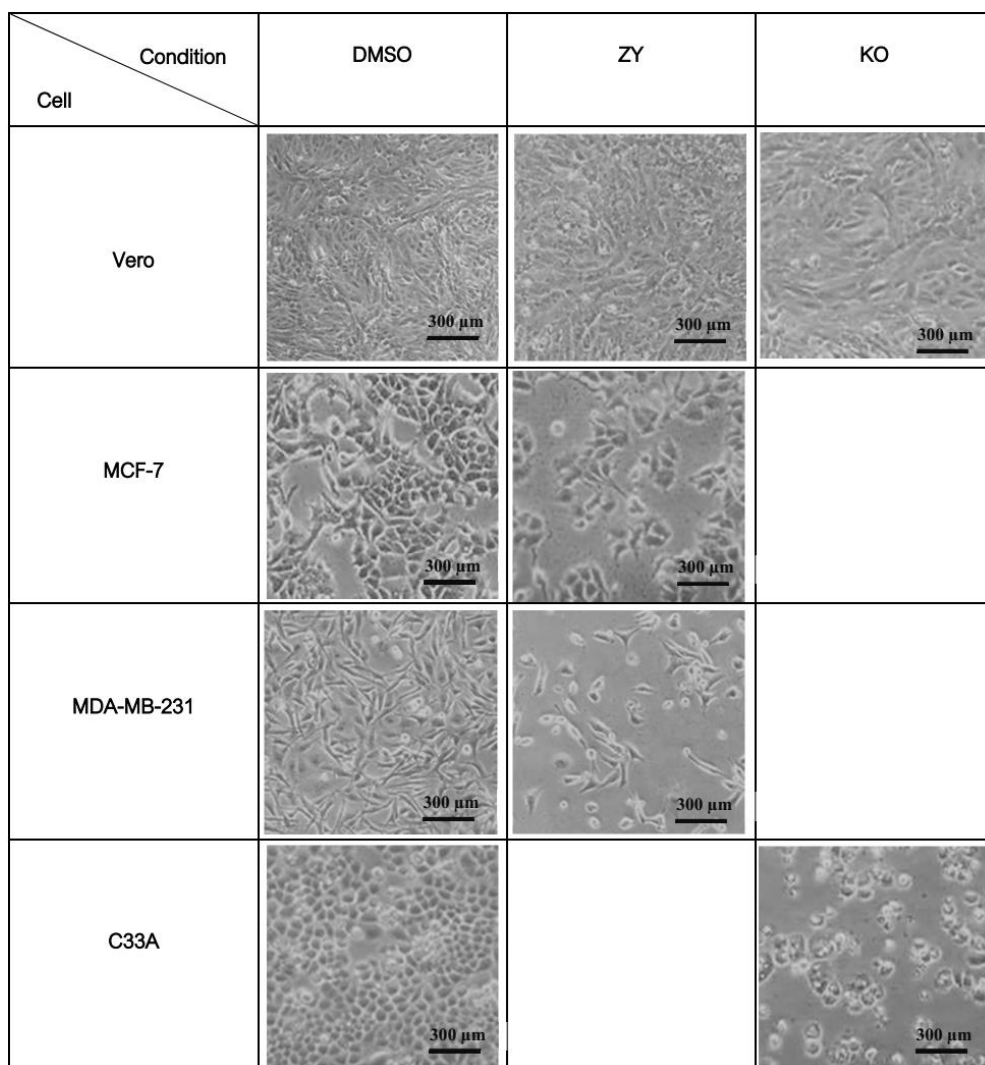


**Figure 1 (cont.)** Cell viability of cancer and non-cancerous cells after treatment with various concentrations of leaf extracts from six cultivars of watermelon for 0, 24, 48, or 72 hours. %Cell viability at 0-h incubation of each concentration was set as 100%.

Moreover, changes in cell morphology and cell number after treatment were also observed under inverted microscope (Figure 2). C33A cells treated with KO extract, as well as MCF-7 and MDA-MB-231 cells treated with ZY extract exhibited the decrease in cell number and the smaller cell size compared to non-treated cells. Obviously, Vero cells with or without treatment remained in the same morphology and colony density. The results indicated the selective anti-proliferative effect of KO and ZY extracts on cancer cells with low toxicity to non-cancerous cells. Hence, further identification of anti-tumor compounds from these active *C. lanatus* leaf extracts and investigation of underlying mechanism are needed.

**Table 1** The IC<sub>50</sub> values (µg/mL) of watermelon extracts against cervical and breast cancer cells at 72-h treatment.

Cell \ Extract	AP	KO	KN	YY	ZY	DN
Vero	>400	>400	>400	>400	>400	>400
MDA-MB-231	>400	>400	>400	>400	400	400
MCF-7	>400	>400	>400	>400	>400	>400
C33A	177	148	132	127	165	166
HeLa	398	185	323	378	369	>400
SiHa	>400	>400	>400	>400	>400	>400



**Figure 2** Morphological changes of cells treated with IC<sub>50</sub> of extracts (ZY, KO) or 0.2% DMSO for 72 hours. Photographs were taken under inverted microscope at 10X objective lens magnification. The scale bar represents 300 µm.

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### Conclusion

Among six cultivars, leaf extracts from Zonya and King orange Thai watermelon showed the most potent inhibitory effect on cervical and breast cancer cells with little effect on non-cancerous cells.

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