Anti-proliferative Activity of Zingiberaceae Crude Extracts against Human Embryonic Kidney Cell Line (HEK 293T/17)

Oradee Khammaneejan[1], Janejira Jaratsittisin[1], Sittiruk Roytrakul[2], Wutigri Nimlamool[3], Siriporn Okonoki[4], Nitwara Wikan[1] and Duncan R. Smith[1,*]

[1]Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhon Pathom, Thailand
[2]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand
[3]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[4]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[*]Corresponding author, E-mail: duncan.smi@mahidol.ac.th

Abstract

Cancer has become the second most common cause of death, and its incidence and mortality rates have increased rapidly worldwide, including Thailand. Despite several types of cancer treatment being available, these treatments are expensive and associated with terrible side effects and eventual resistance. Plant-derived natural products have been developed as anti-cancer drugs because of their safety and efficacy. Several plants in the family *Zingiberaceae* have been reported to possess anti-cancer properties against several types of cancer. This study sought to determine the potential anti-proliferative activity of 9 crude extracts from Zingiberaceae plants towards human embryonic kidney (HEK 293T/17) cells. The extracts were *Zingiber officinale* Roscoe (CE1), *Alpinia galanga* (L.) Willd (CE2), *Curcuma mangga* Valeton & Zijp (CE3), *Curcuma aeruginosa* Roxb. (CE4), *Curcuma longa* L. (CE5), *Boesenbergia rotunda* (L.) Mansf. (CE6), *Kaempferia parviflora* Wallich. ex Baker. (CE7), *Zingiber montanum* (J.Koenig) Link ex A.Dietr. (CE8), and *Zingiber ottensii Valeton* (CE9). The cytotoxic effects of crude extracts were determined by MTT assay. The results showed that all nine crude extracts had a strong cytotoxic effect on HEK 293T/17 cells in a concentration-dependent manner. The 50% cytotoxic concentration (CC_{50}) of CE1-CE9 were 0.228, 0.235, 0.261, 0.230, 0.187, 0.160, 0.190, 0.218, and 0.266 mg/ml, respectively. The most significant cytotoxic effects were seen with CE5 and CE6. These two crude extracts had the lowest CC_{50} and extremely affected morphology of treated cells at low concentrations, suggesting that CE5 and CE6 have the potential to be the candidates for cancer therapeutic agents.

Keywords: *Anti-cancer, Zingiberaceae, HEK 293T/17, Cytotoxic effect*

1. Introduction

Cancer is a term for diseases in which abnormal cells proliferate uncontrollably and can penetrate nearby tissues. Cancer is the primary leading cause of death globally. The incidence and mortality rate of cancers are rapidly growing worldwide, including Thailand. There were an estimated 18.1 million new cancer cases and 9.6 million cancer deaths around the world in 2018 (Bray et al., 2018). Today, there are many types of cancer treatment such as surgery, radiotherapy, and chemotherapy with a variety of different cancer drugs available. The risks of surgery are associated with anaesthesia and infection. Radiotherapy also affects normal cells lying in the radiation field. Using drugs in chemotherapy causes significant side effects and eventual resistance. Besides, hormone therapy, immunotherapy, and gene therapy have been developed for cancer treatment (Abbas and Rehman, 2018). Conventional treatments for some cancers are so expensive (Singleterry, 2017) that many patients could not afford these options.

Plant products are one of the alternatives for the development of anti-cancer drugs that have been ensured to be safe, effective, and less expensive (Yin et al., 2013). The Zingiberaceae family, commonly known as ginger family, consists of 53 genera and over 1200 species (Kress, Prince and Williams, 2002). Several plants in family Zingiberaceae have been reported to be used in traditional medicine for many purposes, including antimicrobial (Nalbantsoy et al., 2008), anti-inflammatory, and anti-oxidant (Rao et al., 1995). Specifically, for anti-cancer, studies have demonstrated that several Zingiberaceae plants had anti-cancer activity against several types of cancer (Kirana et al., 2003). The extract from *Kaempferia parviflora*

(KP), a representative of Zingiberaceae plants, strongly suppressed cell proliferation, cell migration, and invasion and also induced apoptotic cell death in ovarian cancer SKOV3 cells (Paramee et al., 2018). Moreover, KP extract exhibited anti-cancer activity towards HeLa cervical cancer cells (Potikanond et al., 2017). These findings suggest that several plants in the family Zingiberaceae possess anti-proliferative properties. For these reasons, this study aimed to determine the possible anti-proliferative activity of 9 crude extracts from Zingiberaceae plants commonly found in Thailand against cells of the HEK 293T/17 cell line. The results of this study could help in finding potential candidates as new therapeutic agents for cancer treatment.

2. Objective

To evaluate the *in vitro* cytotoxicity of 9 crude extracts from Zingiberaceae plants towards human embryonic kidney (HEK 293T/17) cells.

3. Materials and Methods

3.1 Cell culture

The human embryonic kidney cell line HEK 293T/17 was used in this study. Cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) at 37°C with 5% CO_2.

3.2 Crude extract preparation

In this study, nine crude extracts from Zingiberaceae plants, which are *Zingiber officinale* Roscoe (CE1), *Alpinia galanga* (L.) Willd (CE2), *Curcuma mangga* Valeton & Zijp (CE3), *Curcuma aeruginosa* Roxb. (CE4), *Curcuma longa* L. (CE5), *Boesenbergia rotunda* (L.) Mansf. (CE6), *Kaempferia parviflora* Wallich. ex Baker. (CE7), *Zingiber montanum* (J.Koenig) Link ex A.Dietr. (CE8), and *Zingiber ottensii* Valeton (CE9) were obtained from the Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Thailand. The extracts were prepared from fresh rhizomes which were chopped and extracted with 95% ethanol for 72 hours, followed by concentration and evaporation in a lyophilizer. The crude extracts were dissolved in 100% dimethyl sulfoxide (DMSO) to a final stock concentration of 100 mg/ml and stored at -30°C. Before use, the crude extracts were diluted with DMEM supplemented with 10% FBS into the desired concentration.

3.3 Microscopic observation

HEK 293T/17 cells (5 x 10^5 cells/1 ml/well) were cultured in 12-well tissue culture plates for 24 hours. After removing media, cells were treated with 1 ml of various concentrations of DMSO (0.001-0.5%), or the crude extracts (0.001-0.5 mg/ml), for 24 hours. Cultured cells treated with 10% FBS DMEM was used as the untreated control. Cell morphologies were observed under an inverted microscope after 24-hour post-treatment, and images were captured at 100x magnification.

3.4 MTT assay

HEK 293T/17 cells (6 x 10^4 cells/100 µl/well) were seeded onto 96 well-tissue culture plates for 24 hours. The culture media was removed, and cells were then treated with 100 µl of various concentrations of DMSO (0.001-0.5%), or the crude extracts (0.001-0.5 mg/ml), for 24 hours. Cultured cells treated with 10% FBS DMEM (untreated) was used as a negative control. After that, 12.5 µl of thiazolyl blue tetrazolium bromide (MTT dye) was added into each well. The treated cells were incubated at 37°C for 1 hour. Culture media was removed, and 100 µl of 100% DMSO was subsequently added into each well. The cells were incubated further for another 1 hour, and the absorbance was measured at 570 nm using a microplate spectrophotometer. Each experiment was performed independently in quadruplicate. The 50% cytotoxicity concentration (CC_{50}) and per cent cell viability of each crude extract was calculated from MTT results.

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3.5 Statistical analysis

Data were analyzed by independent-samples t-test and presented as means ± SEM. The *p*-value ($p < 0.05$, $p < 0.01$, and $p < 0.001$) was considered as statistically significant in all analyses.

4. Results and Discussion

Cells were incubated with different concentrations of each crude extract for 24 hours to determine the cytotoxic effects of 9 crude extracts from Zingiberaceae plants on HEK 293T/17 cell line. The CC_{50} and per cent cell viability of each crude extract was determined by the MTT assay. CC_{50} of each crude extract is shown in *Table 1*. The CC_{50} of CE1-CE9 were 0.228, 0.235, 0.261, 0.230, 0.187, 0.160, 0.190, 0.218, and 0.266 mg/ml, respectively. The results of per cent cell viability from MTT assay showed that all nine crude extracts had a strong cytotoxic effect on HEK 293T/17 cells in a concentration-dependent manner, as shown in *Figure 1*. CE1 at 0.5 mg/ml showed a maximum cytotoxic effect, where an approximately 87% reduction of cell viability was observed. CE1 at concentrations below 0.025 mg/ml did not affect cell viability related to cell morphology results (*Figure 2*). Cells treated with CE2 at concentrations over 0.01 mg/ml showed a significant reduction in cell viability, while CE2 at 0.5 mg/ml showed a maximum cytotoxic effect (87% reduction). However, CE2 at concentrations below 0.1 mg/ml did not affect the morphology of treated cells. CE3 and CE6 at concentrations over 0.01 mg/ml significantly reduced per cent cell viability of treated cells, but CE3 and CE6 at concentrations below 0.025 mg/ml did not affect the morphology of treated cells. CE3 at 0.5 mg/ml showed a maximum cytotoxic effect (87% reduction), whereas CE6 at 0.25 mg/ml reduced 90% cell viability. CE4 at 0.5 mg/ml showed a maximum cytotoxic effect (90% reduction). CE4 at concentrations over 0.001 mg/ml showed a significant reduction in cell viability, but, at concentrations below 0.05 mg/ml, did not affect cell morphology. CE5, CE7, CE8, and CE9 at 0.5 mg/ml showed a maximum cytotoxic effect, in which they reduced approximately 90, 90, 86, and 80% cell viability, respectively. CE5, CE7, CE8, and CE9 at concentrations over 0.001 mg/ml showed a significant reduction in cell viability but did not affect cell morphology at concentrations below 0.01 mg/ml. All concentrations of DMSO, used as vehicle control, did not show any cytotoxic effect to cells.

Table 1 The 50% cytotoxicity concentration of 9 crude extracts on HEK 293T/17 cells determined by MTT assay

Crude extract	Common name	Scientific name	CC_{50} (mg/ml)
CE1	Ginger	*Zingiber officinale* Roscoe	0.228
CE2	Galangal	*Alpinia galanga* (L.) Willd	0.235
CE3	White turmeric	*Curcuma mangga* Valeton & Zijp	0.261
CE4	Black turmeric	*Curcuma aeruginosa* Roxb.	0.230
CE5	Turmeric	*Curcuma longa* L.	0.187
CE6	Fingerroot	*Boesenbergia rotunda* (L.) Mansf.	0.160
CE7	Black galingale	*Kaempferia parviflora* Wallich. ex Baker.	0.190
CE8	Phlai	*Zingiber montanum* (J.Koenig) Link ex A.Dietr.	0.218
CE9	Black Phlai	*Zingiber ottensii* Valeton	0.266

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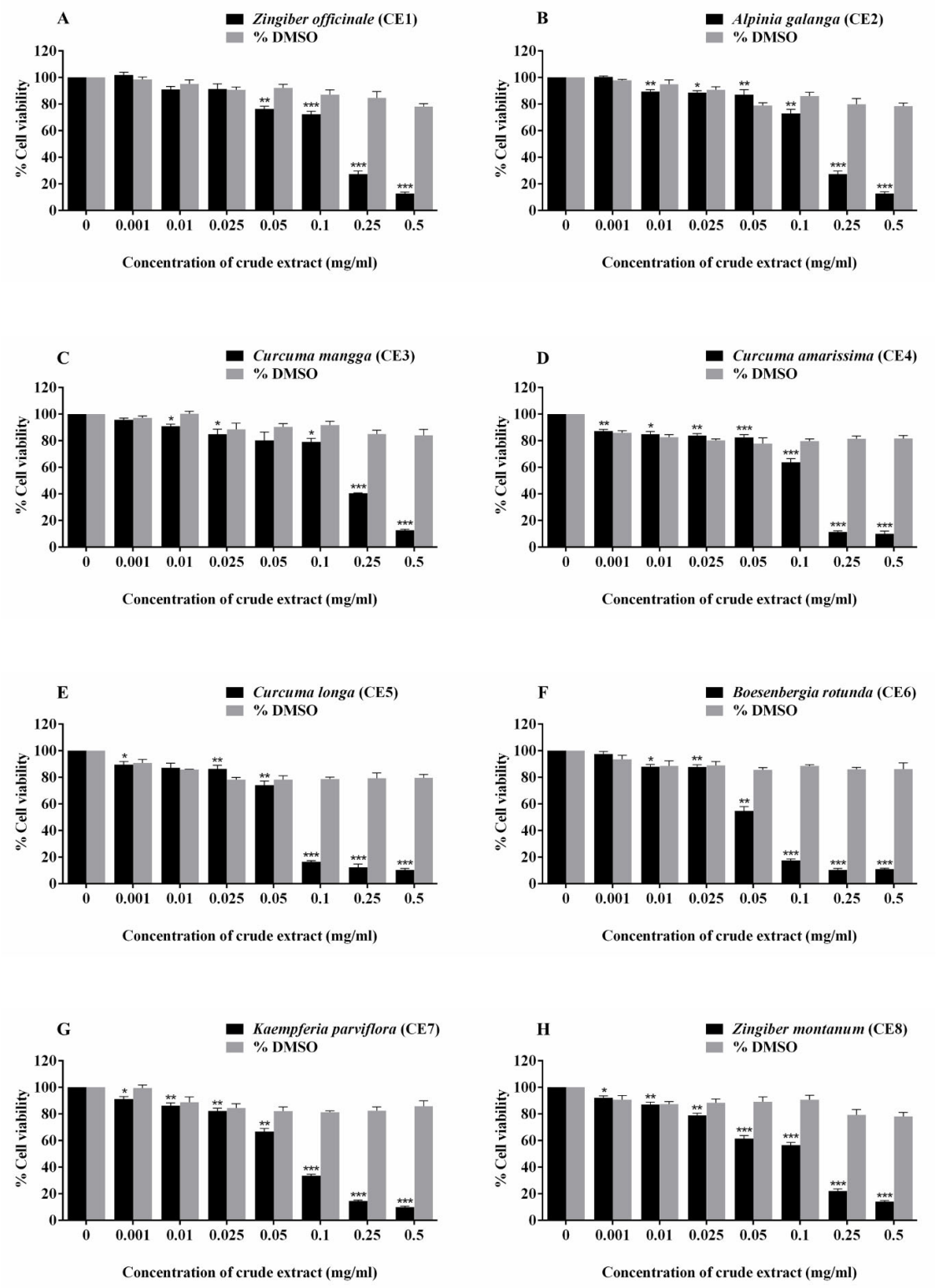

Figure 1 The effects of 9 crude extracts on HEK 293T/17 cell viability (Figure continued on next page)

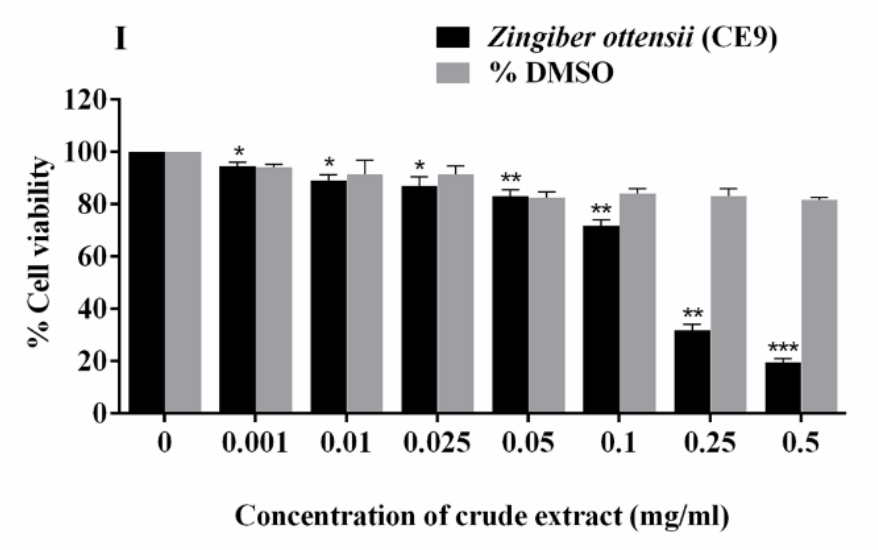

Figure 1 (cont.) The effects of 9 crude extracts on HEK 293T/17 cell viability (Figure continued from the previous page); HEK 293T/17 cells were treated with the indicated concentrations (0.001-0.5 mg/ml) of 9 crude extracts (CE1-CE9) for 24 hours under standard condition. The bar graphs show per cent cell viability assessed by MTT assay. Each experiment was performed independently in quadruplicate and reported as means ± SEM. *p*-value compared to the untreated control (* *p*-value < 0.05, ** *p*-value < 0.01 and *** *p*-value < 0.001). Negative control (0) is plotted.

According to the study of cause-specific mortality in 195 countries around the world from 1980 to 2015, (Wang et al., 2016), the first biggest cause of death is cardiovascular disease, followed by cancer, which is the second leading cause of death globally. Although there are several therapeutic approaches for cancer available, such as surgical removal of the tumour, radiotherapy, chemotherapy, hormone therapy, immunotherapy, and gene therapy, these treatments are expensive and associated with severe adverse side effects to the patients and eventual resistance. At present, many studies have attempted to find new alternative medicines or therapeutic agents for cancer treatment with fewer side effects and more affordable cost. Several plants in family Zingiberaceae have been reported to possess anti-cancer properties against several types of cancer (Kirana et al., 2003).

In this study, nine crude extracts from Zingiberaceae plants (CE1-CE9) were investigated for anti-proliferative activity against HEK 293T/17 cells. The MTT assay was performed to evaluate the cytotoxicity of each crude extract. They were found that all nine crude extracts decreased the cell viability of HEK 293T/17 cells in a concentration-dependent manner with different CC_{50}. The ethanolic extracts of 4 species of Zingiberaceae, which are *Z. officinale* Roscoe (CE1), *C. mangga* Valeton & Zijp (CE3), *C. aeruginosa* Roxb. (CE4), and *C. longa* L. (CE5), were previously screened for antitumor activity against human's breast cancer cell line MCF-7 with CC_{50} of 0.046, 0.044, 0.119, and 0.031 mg/ml, respectively (Kirana et al., 2003). Methanol extracts from rhizomes of *A. galanga* (L.) Willd (CE2) and *B. rotunda* (L.) Mansf. (CE6) were evaluated for their cytotoxicity towards MCF-7 cancer cells with CC_{50} of 0.030 and 0.031 mg/ml, respectively (Zaeoung, Plubrukarn, and Keawpradub, 2005). The CC_{50} value of the extract from *K. parviflora* Wallich. ex Baker. (CE7) on HeLa cervical cancer cells was 0.22 mg/ml (Potikanond et al., 2017). Anti-proliferative activity of *Z. montanum* (J.Koenig) Link ex A.Dietr. (CE8) and *Z. ottensii* Valeton (CE9) has not been reported. The CC_{50} values determined here are different from previous studies. Many factors affect the $CC_{50,}$ including species, tissue and cell type, the confluence of the cells, time of the crud extract treatment, conditions for cell culture such as medium, or even the lineage of the cells.

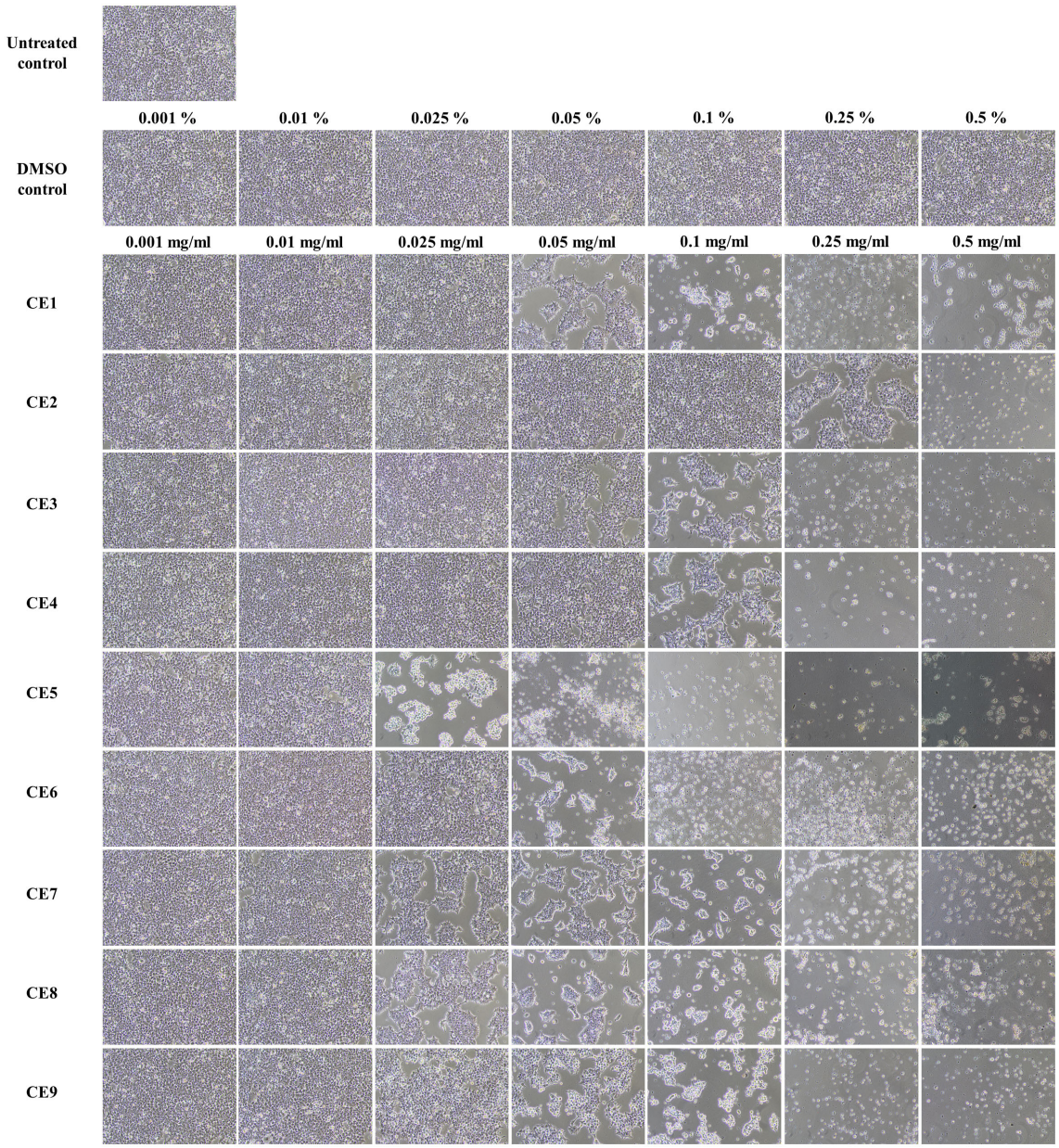

Figure 2 Cell morphology after 24 hours of different concentrations of 9 crude extracts treatment; HEK 293T/17 cells (5 x 10^5 cells/1 ml/well) were cultured in 12-well tissue culture plates for 24 hours. Cells were treated with crude extracts under standard condition with DMSO treated cells as the control. Cell morphologies were observed under an inverted microscope after 24 hours post treatment.

The morphological changes of HEK 293T/17 cells were also observed under an inverted microscope after 24-hour post-treatment of each crude extract at different concentrations. The crude extracts caused cell rounding and detachment from the surface of cell culture plates, which is one of the classic characteristics of apoptosis (Potikanond et al., 2017). The finding suggests that the crude extracts reduced the cell viability of HEK 293T/17 cells via the induction of apoptosis. *K. parviflora* (KP), one of the Zingiberaceae plants, has shown that it strongly inhibited signal transducer and activator of transcription

3 (STAT3) activation and interleukin-6 production in HeLa cervical cancer cells (Suradej et al., 2019). Amongst nine crude extracts, CE5 and CE6 showed the most significant cytotoxic effects to HEK 293T/17 cells. These two crude extracts had the lowest CC_{50} and extremely affected morphology of treated cells at low concentrations. CE5 at 0.5 mg/ml and CE6 at 0.25 mg/ml showed a maximum cytotoxic effect, in which they reduced approximately 90% cell viability.

5. Conclusion

This study demonstrated that all nine crude extracts from Zingiberaceae plants have potential anti-proliferative activity toward HEK 293T/17 cell line. The most significant cytotoxic effects were seen in crude extracts from *C. longa* L. (CE5) and *B. rotunda* (L.) Mansf. (CE6). These crude extracts had the lowest CC_{50} and extremely affected morphology of treated cells at low concentrations. Further studies are suggested in performing more pieces of evidence, such as effects of crude extracts on cell migration, cell invasion and cell apoptosis, to get more reliable results.

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