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Research article

Anticancer effects of aurisin A extracts from *Neonothopanus nambi* on human papillomavirus-infected cervical cancer cells

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Abstract

Cervical cancer is one of the most common cancers in women worldwide. This study determined the effects of aurisin A on cervical cancer cell lines (Hela, CaSki, SiHa), by investigating the molecular mechanisms underlying the effects of aurisin A treatment and the cytotoxic effect of aurisin A. The number of apoptotic cells and nuclear morphological features were observed using flow cytometry and confocal microscopy, respectively. Migration of cancer cells was determined using a wound-healing assay. The cell-cycle distribution was determined using flow cytometry. Expression of proteins related to cell proliferation, cell-cycle arrest and apoptosis was quantified using western blot analysis. Aurisin A had no cytotoxic effect on normal white blood cells. Treatment of the cervical cancer cell lines with aurisin A resulted in inhibition of cell proliferation. Aurisin A inhibited migration of Hela and CaSki cells by suppressing the level of the protein *vascular endothelial growth factor*. Induction of G0/G1 phase arrest in Hela cells was correlated with cyclin D1 and Cdk-4 downregulation. Similarly, S-phase arrest in CaSki cells was due to Cdk-2 downregulation. Notably, aurisin A induced nuclear condensation and fragmentation, a marker of apoptosis in Hela cells, as revealed in increased caspase-9 expression. No tested concentration of aurisin A changed the nuclear morphology or increased the percentage of apoptotic cells in the CaSki cells. This suggested that the Hela and CaSki cells were sensitive to aurisin A based on different mechanisms. These results clearly indicated that aurisin A is a potent agent for the treatment of cervical cancer and merits further trials and research.

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Introduction

Cervical cancer is the third most common cancer of women worldwide (Yee et al., 2013). Persistent infection with human papilloma viruses (HPVs) is strongly associated in the development of cervical cancer (Resnick et al., 1990). Traditional treatments for cervical cancer are surgery or radiotherapy, but survival rates are poor (Shen et al., 2012; Zhang et al., 2016). Furthermore, these treatments may produce side effects and toxicity against normal cells. Thus, there is a clear need for new and effective treatments for cervical cancer that have low toxicity.

HPVs are small, double-stranded, circular DNA viruses that infect the epithelium of the skin at the basal layer (cutaneous HPV types) and mucous tissues (mucosal HPV types) (Zur Hausen, 1996). More than 150 types of HPV are known. For example, high-risk human papillomavirus (HR-HPV) types 16 and 18 are found in invasive cervical cancers (Moody and Laimins, 2010). The viral oncoproteins E6 and E7 are expressed in HR-HPVs and can inactivate the tumor suppressor protein p53 and the cellular retinoblastoma protein pRB, respectively, leading to the development of cervical cancer (Xia et al., 2017). The four treatment options for HPV infection are cryotherapy, surgical removal, laser therapy and loop electrosurgical excision. The only preventative option is vaccination. However, HPV vaccination is not approved by the FDA as an anti-HPV drug, and may not cover all types of cancer associated with HPV infection (Yang et al., 2016).

Aurisin A is an aristolane dimer sesquiterpene isolated from the luminescent mushroom *Neonothopanus nambi* (Marasmiaceae).Aurisin A reportedly exhibits various medicinal properties, including activity against *Plasmodium falciparum* and *Mycobacterium tuberculosis* (Kanokmedhakul et al., 2012). In addition, aurisin A exhibits cytotoxic effects on human small-cell lung cancer and cholangiocarcinoma cell lines (Kanokmedhakul et al., 2012; Boueroy et al., 2020). It has been reported that aurisin A induced cell cycle arrest and apoptosis in human lung cancer cells (Boueroy et al., 2020). However, the anti-cancer effects in other cancers and underlying mechanisms have not been clearly elucidated.

Materials and Methods

Plant material

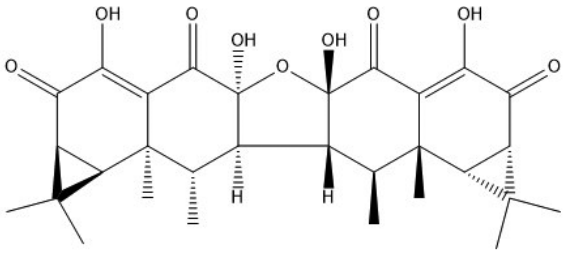
Aurisin A (Fig. 1) was obtained as yellow crystals and extracted from culture liquid of the luminescent mushroom *Neonothopanus nambi* PW1 (Marasmiaceae). The luminescent mushroom was collected from Kok Phutakaarea, Wiang Kao district, Khon Kaen province, Thailand and identified by Prof. W. Saksirirat (Kanokmedhakul et al., 2012). The culture liquid of PW1 (2.025 L) was extracted with EtOAc (2.5 L) and yielded 2.1 g of yellow crystals of aurisin A after crystallization and slow evaporation from EtOAc based on Kanokmedhakul et al. (2012). Its molecular formula $C_{30}H_{36}O_9$ was deduced from HRESITOFMS analysis (*m/z* 540.2257). Aurisin A decomposes at 221.9°C, has a specific rotation, $[\alpha]_D^{26}$+701.3 (*c* 0.1, $CHCl_3$), is soluble in dimethyl sulfoxide (DMSO) at 25 mg/mL and in 95% ethanol (EtOH) at 12.5 mg/mL and has ultraviolet absorption at λ_{max} 331 (logε 4.25) and 268 (logε 4.23) nm (Kanokmedhakul et al., 2012). An amount of aurisin A was dissolved in DMSO to a concentration of 16 mM and further diluted to appropriate concentrations in 1% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) in Dulbecco's modified Eagle medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.).

Human cancer cell lines

The experiments were performed on three human cervical cancer cell lines: SiHa, CaSki and Hela. These cell lines were cultured in DMEM supplemented with 10% FBS, 100 units/mL of penicillin and 100 µg/mL streptomycin (Gibco BRL; Thermo Fisher Scientific, Inc.) at 37°C in 5% CO_2 humidified atmosphere.

Cell viability assays

The effect of aurisin A on the cell viability of various human cancer cell lines (SiHa, CaSki and Hela) was determined according to a sulforhodamine B (SRB) assay. Briefly, cancer cells were seeded into 96-well plates at 37°C. After incubation, the cells were treated with various concentrations of aurisin A for 24 hr, 48 hr and 72 hr. After the treatment, the cells were



Chemical Formula: $C_{30}H_{36}O_9$
Exact Mass: 540.2359
Molecular Weight: 540.6090
m/z: 540.2359 (100.0%), 541.2393 (32.4%), 542.2426 (5.1%), 542.2402 (1.8%)
Elemental Analysis: C, 66.65; H, 6.71; O, 26.63

Fig. 1 Chemical structure of aurisin A

fixed in 10% trichloroacetic acid for 1 hr at 4°C and the plates were then aspirated. SRB dye (Sigma-Aldrich) was added and incubated for 30 min at room temperature. Any unbound dye was removed and the protein-bound dye was solubilized using Tris base solution (10 mM, pH 10) (Sigma). The optical density (OD) was determined at 540 nm using a microplate reader (ELISA Reader; Sunrise).

Cytotoxic effects on normal white blood cells

Human peripheral blood mononuclear cells (PBMCs) were obtained from leftover blood samples at the Blood Bank, Faculty of Medicine, Khon Kaen University, Thailand. Human PBMCs as normal control cells were isolated using the standard Ficoll-hypaque gradient centrifugation method (Clément et al., 1998). Utilization of these specimens had been approved by the Center for Ethics in Human Research, Khon Kaen University, Thailand (HE641322). The effect of aurisin A on the cell viability of normal white blood cells at different concentrations (0 µM, 6 µM, 12 µM, 24 µM, 96 µM, 192 µM, 394 µM) and different time periods (24 hr, 48 hr, 72 hr) was determined based on SRB assay.

Wound-healing assay

Cells at a density of 3×10^5 cells/well were seeded into 6-well plates and grown to 80–90% confluence. The monolayer of cancer cells was wounded by scratching it with a sterile pipette tip, then rinsed with 1× phosphate-buffered saline (PBS) to remove cell debris. The cells were treated with various concentrations of aurisin A (at IC_{30}, IC_{50} and IC_{80}, where IC is the inhibitory concentration at 30%, half maximal and 80%, respectively) in medium containing 1% FBS for periods appropriate to wound closure. The extent of wound healing was determined and photographed.

Cell-cycle analysis

Cells (1×10^5 cells/well) were seeded into 6-well plates. On the following day, the cells were treated with aurisin A at different concentrations for 24 hr. The cell suspensions were fixed with 70% ethanol at 4°C, treated with RNase A (final concentration 2 µg/mL) for 30 min and stained with propidium iodide (PI) at a final concentration of 2.4 µg/mL at room temperature. The stained DNA was examined using a FACScanto™ II flow cytometer (Becton Dickinson) and the DNA content determined using the FACS Diva™ software (Becton Dickinson).

Acridine orange-ethidium bromide staining

Characteristic apoptotic features were visualized using fluorescence staining with acridine orange and ethidium bromide. The cancer cells were treated with different concentrations of aurisin A for 24 hr and stained with 100 µg/mL acridine orange-ethidium bromide mixture (Sigma). The stained cells were observed under a confocal microscope.

Annexin V/PI apoptosis assay

The cancer cells were treated with aurisin A for 24 hr. Apoptotic cells were determined using the Annexin V-FLUOS staining kit (Roche Diagnostic) according to the manufacturer's instruction. After the incubation, both detached and attached cells were harvested. The cells were mixed in 100 µL incubation buffer containing 2 µL Annexin V-FITC and 2 µL PI and incubated at room temperature in the dark. Cells were examined using a FACScanto™ II flow cytometer and the data were analyzed using the FACS Diva™ software.

Western blot analysis

Cells (1×10^6 cells/well) were seeded into 10 cm diameter dishes (Costar; Corning Incorporated) and treated with various concentrations of aurisin A for 24 hr. The cells were washed with 1×PBS and lysed at 4°C for 1 hr with cold RIPA buffer (50 mM Tris-HCl, pH 7.5, 0.5% Nonidet P-40, 150 mM NaCl, 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium dodesyl sulfate, 0.5% deoxycholate). The cell lysates were homogenized and centrifuged at 13,000 revolutions per minute for 30 min at 4°C. Protein concentrations were measured using the Coomassie protein assay kit (Pierce; Thermo Fisher Scientific, Inc.). Cell lysates (5 µg of protein per lane) were fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12%). The proteins were transferred onto a nitrocellulose membrane. The membrane was blocked and probed with primary antibody. The membrane was incubated with primary antibody at 4°C overnight against the VEGF (*vascular endothelial growth factor*), the EGFR (epidermal growth factor receptor), cyclin D1, Cdk-4, Cdk-2, caspase-9 and β-actin; then, the samples were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 hr. The bound secondary antibodies on the nitrocellulose membrane were visualized using an enhanced chemiluminescence reagent (Pierce ECL; Thermo Fisher Scientific), quantified

using densitometry (ImageQuant LAS 4000; GE Healthcare) and analyzed using the Scion Image program (Scion Corp.). The results were expressed as the relative density of protein normalized to β-actin.

Statistical analysis

Data were represented as the mean ± SD of three independent experiments. Statistical comparisons of responses of the control and treated cells to the various experimental conditions were performed using a Student's t test with the SPSS statistical software, version 16.0 (SPSS). Differences between the groups treated with aurisin A and with DMSO was considered significant at $p < 0.05$.

Results

Cytotoxic effect of aurisin A on various cervical cancer cells and normal white blood cells

The three cervical cancer cell lines (Hela, CaSki and SiHa) were treated with various concentrations of aurisin A (0 μM, 1 μM, 2 μM, 4 μM, 8 μM, 16 μM, 32 μM) for 24 hr, 48 hr and 72 hr. Inhibition of proliferation occurred in a dose- and time-dependent manner for the Hela and CaSki cells (Fig. 2, Table 1). The IC_{50} values of Hela and CaSki after treatment with aurisin A for 24 hr were 6.65 ± 0.36 μM and 6.27 ± 0.51 μM, respectively. These two cervical cancer cell lines were more sensitive to this drug than the SiHa cells. Aurisin A at concentrations of 0 μM, 6 μM, 12 μM, 24 μM, 96 μM, 192 μM and 394 μM had no cytotoxic effect on normal white blood cells (Fig. 3).

Table 1 Half maximal inhibitory concentration (IC_{50}) values of aurisin A-treated cervical cancer cell lines at different times

Cell line	IC_{50} (μM)		
	24 hr	48 hr	72 hr
Hela	6.65±0.36	3.38±0.51	< 1
CaSki	6.27±0.51	2.17±0.14	1.36±0.23
SiHa	> 32	> 32	> 32

Values = mean ± SD of three independent experiments

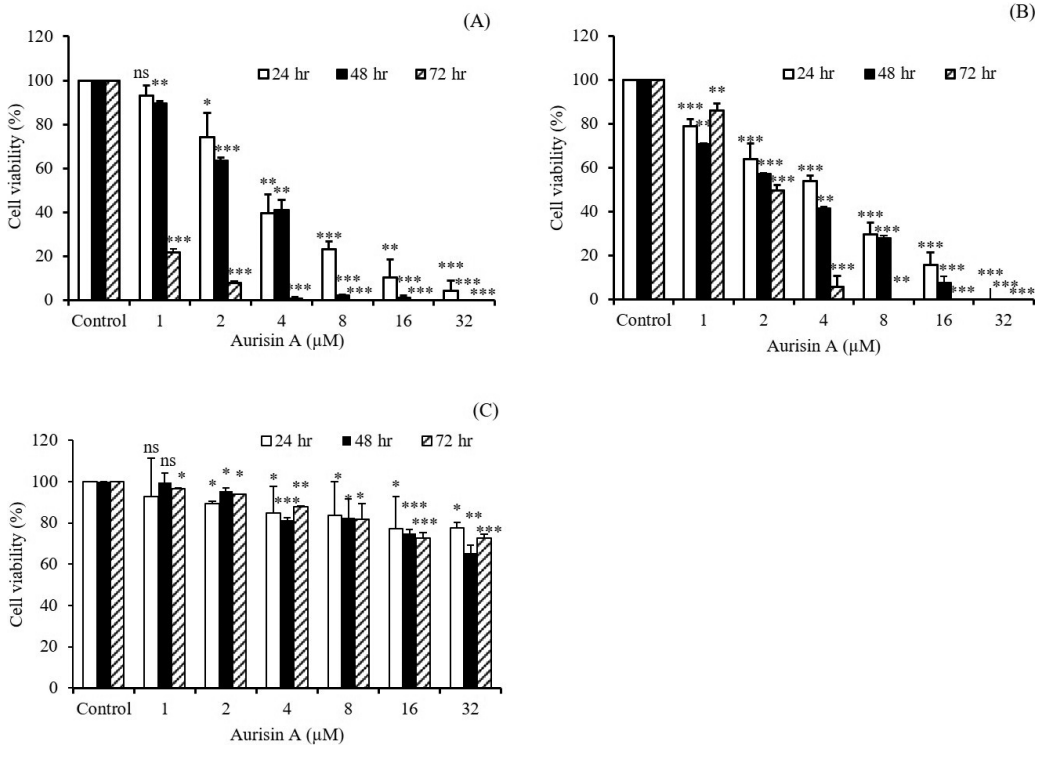


Fig. 2 Cytotoxic effect of different concentrations of aurisin A on cell viability after different treatment times of cells of: (A) Hela; (B) CaSki; (C) SiHa. Histograms represent mean of three independent experiments and error bars = ± SD. Comparisons were made between mean of each aurisin A treated group with the control (dimethyl sufoxide-treated cells) of the same treatment duration. ns = not significant ($p > 0.05$), * = significant ($p < 0.05$), ** = significant ($p < 0.01$), *** significant ($p < 0.001$)

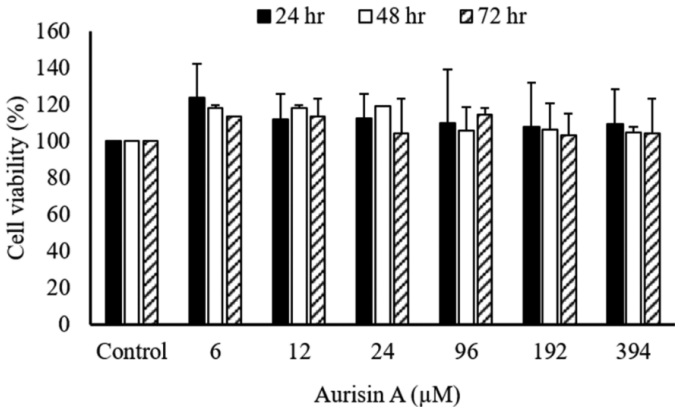


Fig. 3 Effect of different concentrations of aurisin A on white blood cell viability after different treatment times, where histograms represent mean of three independent experiments and error bars = ± SD; The comparisons between control (dimethyl sulfoxide-treated group) and each aurisin A treated group of the same treatment duration are not significantly ($p > 0.05$) different.

Aurisin A inhibition of cell migration in various cancer cells

Two cervical cell lines (CaSki, and Hela cells) were treated with aurisin A to investigate the migration of cancer cells using a wound-healing assay and western blot analysis to examine the expression of the *vascular endothelial growth factor* (VEGF) and the epidermal growth factor receptor (EGFR). As shown in Fig. 4A and 4B, aurisin A at IC_{30}, IC_{50} and IC_{80} concentrations significantly delayed the movement of Hela and CaSki cells into the wound area relative to the controls after treatment for 48 hr and 30 hr, respectively. The percentages of migration area in the Hela and CaSki cells after scratching were significantly decreased compared to the control cells (Fig. 4C). Aurisin A significantly decreased expression of the VEGF in Hela and CaSki cells (Fig. 4D) and down-regulated EGFR expression in CaSki cells (Fig. 4D), but not in Hela cells (Fig. 4D).

Aurisin A-induced cell-cycle arrest in human cancer cells

The mechanisms underlying inhibition of cell proliferation were assessed using flow cytometry and western blot analysis. Two cervical cancer cell lines, Hela and CaSki, were treated with various concentrations of aurisin A for 24 hr. Flow cytometry analysis showed that aurisin A induced significant changes in the cell-cycle distribution in these cells (Fig. 5). As shown in Fig. 5A and 5B, Hela cells were treated with 3.99 μM, 6.65 μM and 10.64 μM (IC_{30}, IC_{50} and IC_{80}, respectively) of aurisin A for 24 hr. There was a significant dose-dependent increase in the percentages of cells in the G0/G1 phase (from 65.8±2.38% to 73.97±0.78%) and a decrease in cells in both the S phase (from 18.5±1.26% to 14.13±0.47%) and the G2/M phase (from 15.57±1.84% to 10.4±0.44%). After the CaSki cells were treated with 3.76 μM, 6.27 μM and 10.03

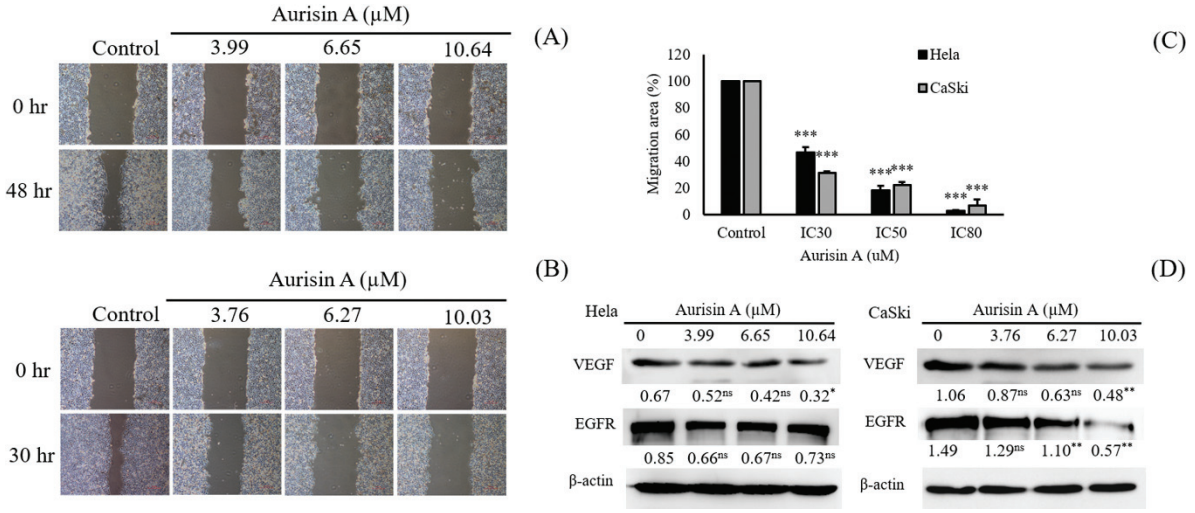


Fig. 4 Effect of aurisin A on cell migration of Hela and CaSki cells after treatment with various concentrations of aurisin A for: (A) 48 hr; (B) 30 hr; (C) distance of migration area (histograms = mean, error bars = ± SD) in Hela and CaSki cells after scratching in three independent experiments and comparisons were made between mean of each treatment group with mean of the respective control (dimethyl sulfoxide treated) where ns = not significant ($p > 0.05$), * = significant ($p < 0.05$), ** = significant ($p < 0.01$), *** = significant ($p < 0.001$); (D) western blot analysis of cell migration-related molecules, VEGF (*vascular endothelial growth factor*), EGFR (epidermal growth factor receptor) in Hela and CaSki cells, where each protein's expression is normalized relative to β-actin; numbers below bands represent mean value of the relative intensity.

μM (IC_{30}, IC_{50} and IC_{80}, respectively) of aurisin A for 24 hr, the percentages of cells in the S phase significantly increased (from 12.87±0.27% to 16.23±0.57%), while the percentages significantly decreased in the G0/G1 phase (from 74.3±0.2% to 71.1±0.89%) and G2/M phase (from 12.63 ± 0.23% to 11.53±0.29%), as shown in Figs. 5A and 5B. Treatment with aurisin A decreased the expression of cyclin D1 and Cdk-4 in the Hela cells and decreased Cdk-2 in the CaSki cells, all of which are important for cell-cycle regulation (Figs. 5C and 5D).

Morphological changes and nuclear condensation induced by aurisin A

Homogenous distribution of chromatin was observed in untreated cells, whereas treatment with aurisin A for 24 hr resulted in a bright green appearance and condensation and fragmentation of nuclei in the Hela cells (Fig. 6). The changes in nuclear morphology after aurisin A treatment were characteristic of apoptosis in the Hela cells. However, aurisin A did not affect the nuclear morphology in the CaSki cells (Fig. 6).

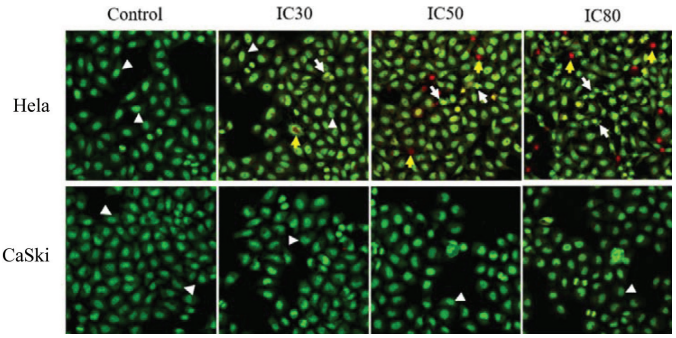


Fig. 6 Nuclear morphology (acridine orange-ethidium bromide stained) of Hela and CaSki cells after being treated with aurisin A for 24 hr, where nuclear condensation and fragmentation of cells indicate early apoptotic cells (white arrows), late apoptotic cells showing loss of membrane integrity indicated by a red color (yellow arrows), living cells are uniformly stained green (white arrowheads) and IC30, IC50 and IC80, are the inhibitory concentrations at 30%, half maximal and 80%, respectively

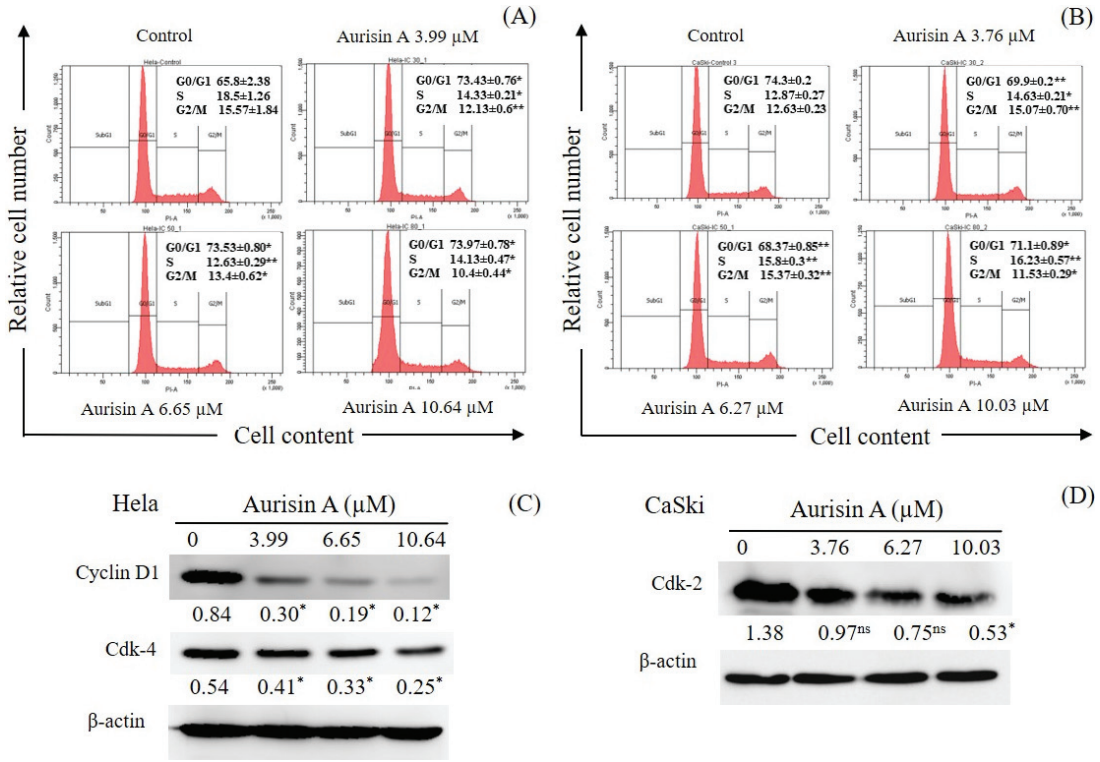


Fig. 5 Effect of different concentrations of aurisin A on cell-cycle distribution in Hela and CaSki cells after being treated for 24 hr: (A) DNA content of Hela; (B) DNA concentration of CaSki; (C) Western blot of cell cycle arrest-related molecules, cyclin D1, Cdk-4 and Cdk-2 in Hela cells; (D) in CaSki cells, where each protein expression is normalized relative to β-actin, number under each protein band in (C) and (D) represents mean value of the relative intensity, an asterisk indicates significant ($p < 0.05$) difference between relative intensity of aurisin A- and control (dimethyl sulfoxide-treated) group and ns = not significant ($p > 0.05$)

Aurisin A-induced apoptosis in human cancer cells

Consistent with the nuclear morphological observations, the percentages of Hela cells that became apoptotic after exposure to aurisin A at 3.99 µM, 6.65 µM and 10.64 µM for 24 hr increased to 10.0±2.43%, 10.25±0.21% and 14.63±2.66%, respectively, with the last value being significantly greater than in the controls (Figs. 7A and 7C). Supporting this observation, western blot revealed that aurisin A treatment significantly increased the apoptosis-related protein caspase-9 in the Hela cells (Fig. 7D). There were no significant increases in the percentages of apoptotic CaSki cells on exposure to aurisin A at 3.76 µM, 6.27 µM and 10.03 µM (Figs. 7B and 7C).

Discussion

The present study indicated that aurisin A is one of the candidate anticancer compounds that displays growth inhibition in a dose-dependent manner with different IC_{50} values in both the cervical cancer (Hela and CaSki) cell lines, while the SiHa cells were less sensitive to the drug. In addition, the selectivity effect of aurisin A was exhibited by against cancer cancer cell lines compared to peripheral blood mononuclear cells. Kanokmedhakul et al. (2012) reported that aurisin A acted against *Plasmodium falciparum* (IC_{50} value of 0.80 µM) and against *Mycobacterium tuberculosis* (minimum inhibitory concentration (MIC) value of 92.55 µM).

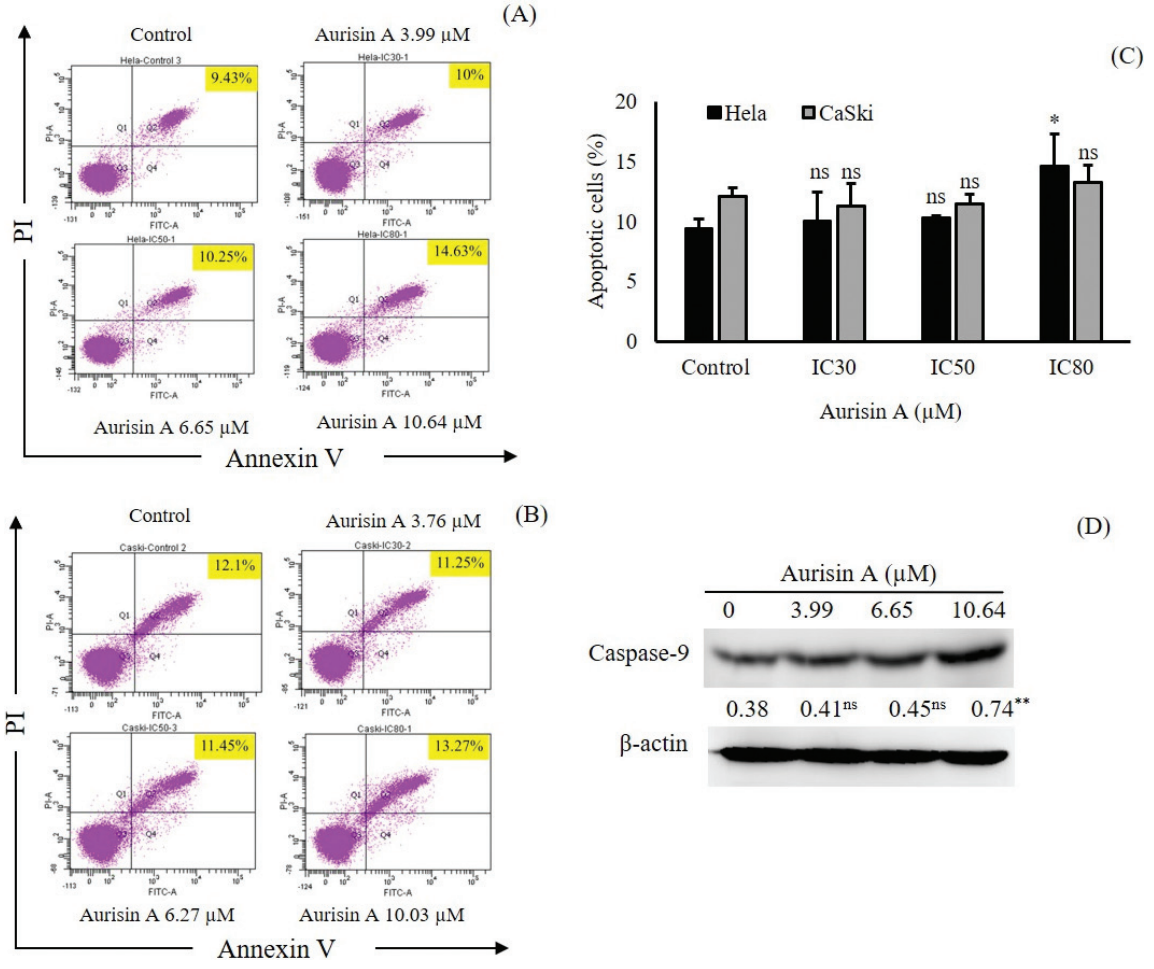


Fig. 7 Effect of different concentrations aurisin A on induction of apoptosis in Hela and CaSki cells after being treated for 24 hr: (A) Hela cells after Annexin V-PI staining; (B) CaSki cells after Annexin V-PI staining; (C) percentage of apoptotic cells in Hela and CaSki cells (histograms = mean, error bars = ±SD); (D) western blot of apoptosis-related molecules, caspase-9 in Hela cells, where protein expression is normalized relative to β-actin, number under each protein band represents mean value of relative intensity, ** = significant ($p < 0.01$) difference between relative intensity of aurisin A-treated and control (dimethyl sulfoxide-treated group) and ns = not significant ($p > 0.05$)

Furthermore, aurisin A was cytotoxic against the human small-cell lung carcinoma cell line NCI-H187 (IC_{50} value 1.55 µM), the human breast cancer cell line BC1 (IC_{50} value 3.72 µM) and cholangiocarcinoma cell lines including KKU-100, KKU-139, KKU-156 and KKU-213 with IC_{50} values of 2.77, 1.83, 1.57 and 1.75, respectively (Kanokmedhakul et al., 2012).

The viral oncoproteins E6 and E7 are the main oncoproteins inducing cervical cancer. These viral oncoproteins can interfere with cell-cycle pathways by their effects on cell-cycle-regulated proteins. E6 degrades p53 while E7 inactivates the function of pRB. E7 can also bind the cyclin/CDK inhibitor $p21^{CIP}$ and destroy its ability to inhibit the function of cyclin/CDK complexes (Jones et al., 1997). The cell-cycle pathway is biologically important for the development and progression of cervical cancer. Most chemo-preventative drugs control the growth of cancer cells either by arresting the cell cycle phase or by induction of apoptosis by p53-dependent or independent mechanisms (Sa and Das, 2008). The cell cycle progression of eukaryotic cells is regulated by cyclin and Cdk (King and Cidlowski, 1998). The G0/G1 phase is regulated by the cyclin D1-Cdk-4 complex and transition from G1 to S is regulated by cyclin E-Cdk2 (King and Cidlowski, 1998). The present study demonstrated that aurisin A had a strong effect on cell-cycle arrest in the G0/G1 and S phases in Hela and CaSki cells, respectively, and was correlated to decreased expression of cell-cycle-regulated proteins (cyclin D1, Cdk-4 and Cdk-2). The results suggested that treatment with aurisin A induced cell-cycle arrest in cervical cancer and were in accordance with a study that found an essential oil extract from *Curcuma wenyujin* (CWE) and the bioactive ingredients of CWE are sesquiterpenes, where CWE induced cell cycle arrest in the G1 phase in cervical cancer cells that was mediated through decreased expressions of cyclin D1, cyclin D3, Cdk-4 and Cdk-6 (Lim et al., 2010). A new sesquiterpene, (Z)-7-acetoxy-methyl-11-methyl-3-methylene-dodeca-1,6,10-triene, from extracts of hairy root cultures of *Artemisia annua* inhibited human lung carcinoma 95-D cells by cell cycle arrest in the G1 phase (Zhai et al., 2010).

Apoptosis is an important mechanism of drug treatment for cancer (Danial and Korsmeyer, 2004). Apoptosis through the mitochondrial pathway can be induced by increasing the Bax:Bcl-2 ratio and triggering the release cytochrome *c* from mitochondria into the cytoplasm (Fulda and Debatin, 2006). Cytochrome *c* will form a complex with procaspase-9 and Apaf-1, leading to activation of caspase-9 and caspase-3, and apoptotic cell death (Fulda and Debatin, 2006). The present study suggested that aurisin A induced apoptosis by regulating caspase-9 in Hela cells. The current data are supported by other studies, in which multiple anti-cancer drugs induced cancer cell apoptosis by inducing caspase-9 (Srinivas et al., 2003; Peng et al., 2015). Gallic acid inhibits Hela cells through induction of apoptosis (You et al., 2010). In the present study, aurisin A also induced apoptosis in Hela cells as found by Annexin V/PI staining and the detection of apoptosis-related proteins by western blot. However, no tested concentration of aurisin A changed the nuclear morphology or increased the percentage of apoptotic cells in CaSki cells. This suggested that Hela and CaSki cells were sensitive to aurisin A via different mechanisms. The anti-cancer activity of aurisin A may be via the inhibition of cancer cell migration and the induction of cell-cycle arrest or apoptosis or both. Therefore, aurisin A exerted its anti-cancer properties in each of the cervical cancer cell lines via a different mechanism. This was in agreement with a study that reported the effect of the aqueous extract of *Ficus religiosa* acted via different mechanisms on the induction of cell cycle arrest in the G1/S phase compared to the induction of apoptosis in SiHa cells, while activation of apoptosis in Hela cells was not via induction of cell cycle arrest (Choudhari et al., 2013). In addition, the aqueous extract of *Ficus religiosa* did not induce any cytotoxic effect on the growth of C33A (Choudhari et al., 2013). Meissner (1999) reported that Hela contains around 1–2 integrated copies of the HPV 16 genome, CaSki has 60–600 integrated copies of the HPV 16 genome and SiHa contains 1–2 integrated copies of the HPV 18 genome. The altered response of each cervical cancer cell line after treatment could depend on their genetic make-up that includes their HPV status, the viral copy number (Meissner, 1999) and the rate of replication in different cell lines (Choudhari et al., 2013). In the present experiment, the response of aurisin A treatment differed in SiHa, CaSki and Hela cells.

The viral oncoprotein E6 can induce migration in cervical cancer by down regulation of miR-23b, resulting in upregulation of uPA (Yeung et al., 2011). The VEGF is an important prognostic marker for various cancers such as colorectal cancer, lung cancer, ovarian cancer and cervical cancer (Tsai et al., 2013; Gkiozos et al., 2015; Cheng et al., 2013; Zhang et al., 2017). The VEGF is involved in the control of angiogenesis by modulation of endothelial cell growth and migration (Evensen et al., 2009; Ferrara, 2004). The HPV oncoproteins activate angiogenesis of cervical cancer by increased expression of the VEGF (Toussaint-Smith et al., 2004). The present study identified that aurisin A inhibited cancer cell migration by decreasing the expression of the VEGF in Hela and CaSki cells. The EGFR and its ligand are normally expressed in

cervical cancers (Kulkarni et al., 2001), but in the present study decreased only in the CaSki cells treated with aurisin A, not in the Hela cells. Furthermore, aurisin A induced apoptosis in Hela cells as evidenced by the upregulation of caspase-9 protein which is a regulated mitochondrial apoptotic pathway (Fulda and Debatin, 2006). Consistent with the present study, CME induced apoptosis in cervical cancer by caspase-3, 6, 7, 9 activations (Zhai et al., 2010). In further agreement with the present study, the anticancer effect of emodin (1,3,8-trihydroxy-6-methylanthraquinone) from *Polygonum cuspidatum* induced apoptosis by caspase-9, caspase-3 and cleavage of poly (ADP-ribose) polymerase activations in cervical cancer cells (Srinivas et al., 2003). Furthermore, the flavonoid quercetin induced mitochondria-mediated apoptosis in Hela cells (Priyadarsini et al., 2010).

In conclusion, the present study suggested that aurisin A induced apoptosis and cell cycle arrest and inhibited cancer cell migration which may useful for anticancer development.

Ethics Statements

The study protocol (previous sample collection) was approved by The Human Research Ethics Committee, Khon Kaen University, Thailand (approval no. HE641322).

Conflict of Interests

The authors declare that there are no conflicts of interest.

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