



UNIVERSITI PUTRA MALAYSIA

**SUPPRESSION EFFECTS OF *PANDANUS AMARYLFOLIUS* AND
STROBILANTHES CRIPUS EXTRACTS ON THE GROWTH OF BREAST
CANCER CELLS BY INDUCING p53-MEDIATED APOPTOTIC PATHWAY.**

CHONG HUEH ZAN

FPSK(p) 2010 4

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By

CHONG HUEH ZAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in

Fulfillment of the Degree of Doctor of Philosophy.

May 2010

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of
the requirement for the degree of Doctor of Philosophy

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Pandanus amaryllifolius (PA) or commonly known as daun pandan is a common culinary plant in South-east Asia from the screw pine family. The leaves of PA are added to cooking to give a pleasant nutty aroma, reminiscent to fresh hay. Besides for aromatic value, PA has a long history in local traditional medicine system alleviating ailments and promotion of well-being. Meanwhile, *Strobilanthes crispus* ZII 109 (L) Bremek (Acanthaceae) is a native plant to countries from Madagascar to Indonesia. *Strobilanthes crispus* (SC) or pecah beling has long been used as medicinal plants against various conditions and cancer.

In this research, chemopreventive properties of *Pandanus amaryllifolius* and *Strobilanthes crispus* extracts were investigated via screening against a panel of human cancer cell lines and the normal fibroblast cells to screen for selective cytotoxicity and anti-proliferative

activity. Both ethanolic PA and SC extracts were found to display selective cytotoxicity and anti-proliferative against breast cancer cells but not on normal cells. In the sample treated with PA for 72 hours, MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that cytotoxicity activity of PA was found to be effective against non-hormone dependent breast cancer cell lines MDA-MB-231, 50% inhibition of cell growth at a concentration of ($IC_{50}=90\text{ }\mu\text{g/mL}$). Anti-proliferative activities of PA in MDA-MB-231 cells were further evaluated using the colorimetric pyrimidine analogue BrdU incorporation. Exposure of PA extract at IC_{50} concentration ($90\text{ }\mu\text{g/mL}$) resulted in a decrease in percentage of cells that underwent DNA synthesis in the cell cycle (S phase). Therefore, suggesting PA extract inhibited proliferation of MDA-MB-231. Further investigation of chemoprevention activities focusing on modulations of cell cycle events and induction of apoptosis was carried out. Flow-cytometry cell cycle RNAase/PI assay of MDA-MB-231 cells treated with PA extract ($90\text{ }\mu\text{g/mL}$) revealed alteration in cell cycle distribution and accumulation of cell population at G1 phase. G1 arrest of treated MDA-MB-231 cells was found to involve upregulation of cyclin dependent kinase inhibitors (CKI) p21 protein level and in the inhibition of cdk2 and 4 activities. At 48 and 72 hr experimental time point, exposure of PA ($90\text{ }\mu\text{g/mL}$) towards MDA-MB-231 cells was found to induce cell death. Apoptosis event was assessed by various assays portraying different apoptotic features. Detection of apoptosis was carried out by Annexin V/FIT-C staining, Acridine orange/Propidium iodide staining as well as DNA ladder assay and was confirmed using TUNEL assay. Apoptosis induction was found to involve activation of caspase cascades and release of Cytochrome C. The molecular mechanisms in the induction of apoptosis by PA in MDA-MB-231 cells were found to

involve upregulation of tumour suppressor protein p53 and pro-apoptotic bax protein while a reduction in the expression of inhibitor of apoptosis XIAP protein.

On the other hand, exposure of SC extract (30 µg/mL) resulted in 50% inhibition of cell growth in hormone dependent breast cancer cell line MCF-7. Further analysis indicated the presence of subG1 population in MCF-7 cells treated with SC extract (30 µg/mL), a classical feature of apoptotic cells. Detection of apoptotic MCF-7 cells was also apparent in flow cytometry Annexin V/FIT-C staining and via detection of double or single DNA break strands in TUNEL assay. Mitochondrial activated apoptosis induction by SC in MCF7 cells was found to involve activation of caspases and release of Cytochrome C into the cytosol thus, activating initiator and effector caspase 3/7. Upregulation of tumour suppressor p53 protein was detected upon SC exposure however, apoptosis induction in treated MCF-7 cells was found to be p53 transcriptive independent as pro-apoptotic bax and Bcl-2 protein were not activated upon activation of apoptosis machinery.

In this research, the targeted modulation or restoration of the intracellular signaling network by *Pandanus amaryllifolius* and *Strobilanthes cripus* extracts towards breast cancer cells offered a potential strategy in preventing abnormal cell proliferation and promoting cell death of neoplastic cells in an *in-vitro* model.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

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Pandanus amaryllifolius (PA) atau daun pandan ialah tumbuhan dari famili *screw pine* yang sering diaplikasi ke dalam sajian orang Asia tenggara untuk menambah keharuman dan kelazatan ke dalam masakan. Selain kegunaan dalam aplikasi sajian, daun pandan turut mempunyai nilai perubatan dalam sejarah perubatan traditional. Daun pandan dikatakan mampu mengubati pelbagai penyakit dan ekstrak daun pandan juga diambil sebagai kaedah pencegahan penyakit dan mengekalkan kesihatan. *Strobilanthes crispus* ZII 109(L.) Bremek pula ialah sejenis tumbuhan dari famili *Acanthaceae*. *Strobilanthes crispus* (SC) atau lebih dikenali dengan nama pecah beling boleh didapati di sekitar Madagascar sehingga ke Indonesia. Pecah beling mempunyai nilai perubatan yang tinggi dan diguna sebagai rawatan untuk kanser dan pelbagai masalah kesihatan yang lain.

Dalam kajian penyelidikan ini, daun pandan (PA) dan pecah beling (SC) disaring untuk keberkesanan mereka dalam proses perencatan pertumbuhan sel-sel kanser serta kesan sitotoksik secara *in-vitro* ke atas sel-sel titisan kanser dan sel normal fibroblast. Ekstrak ethanol dari daun pandan (PA) dan pecah beling (SC) didapati mampu merencat proliferasi sel kanser payudara tetapi tidak mempunyai kesan sitotoksik ke atas sel normal. Dalam analisis 72 jam MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ekstrak PA didapati membantut pertumbuhan sel kanser payudara MDA-MB-231 dengan nilai IC_{50} 90 μ g/mL (konsentrasi yang merencat pertumbuhan sel kanser sebanyak 50%). Ekstrak PA juga didapati merencat peratusan sel kanser payudara MDA-MB-231 yang melalui fasa sintesis (S phase) dalam ujian kolorimetric pyrimidine analog BrdU. Dalam ujikaji flow cytometer RNAase/PI, ekstrak PA (90 μ g/mL) ke atas sel kanser payudara MDA-MB-231 menunjukkan kesan perencatan pertumbuhan ke atas proses kitaran sel serta menyebabkan pambatutan proses kitaran sel di fasa G1. Pembantutan proses kitaran sel MDA-MB-231 pada fasa G1 adalah kerana kesan modulasi ke atas protein *cyclin dependent kinase inhibitors* (CKI) p21 yang merencat aktiviti cdk2 dan 4 dalam proses kitaran sel. Pada 48 dan 72 jam selepas pendedahan ekstrak PA (90 μ g/mL) mengakibatkan kematian sel pada MDA-MB-231 secara apoptosis. Kematian sel secara apoptosis telah dinilai melalui ujikaji Annexin V/FIT-C, Acridine orange/Propidium iodide *staining*, DNA ladder dan TUNEL. Proses apoptosis ke atas sel MDA-MB-231 didapati melibatkan pengaktifan protein caspase dan Cytochrome C. Mekanisme apoptosis sel kanser MDA-MB-231 di peringkat molekular didapati melibatkan peningkatan protein *tumour suppressor protein* p53 dan protein bax

yang menyumbang ke arah induksi apoptosis. Selain itu, ekstrak PA juga menyebabkan penurunan ekspresi protein *inhibitor of apoptosis* XIAP.

Manakala, ekstrak SC pada konsentrasi 30 µg/mL turut menyebabkan perencatan 50% ke atas proliferasi sel kanser payudara MCF-7. Ekstrak SC (30 µg/mL) juga didapati mengakibatkan kejadian populasi *subG1*, satu ciri klasik yang menunjukkan kesan induksi apoptosis ke atas sel MCF-7. Kematian sel MCF-7 secara apoptosis telah dinilai melalui ujikaji Annexin V/FIT-C, Acridine orange/Propidium iodide *staining*, DNA *ladder* dan TUNEL. Proses apoptosis ke atas sel MCF-7 juga didapati melibatkan pengaktifan protein caspase dan Cytochrome C ke dalam sitosol sel. Mekanisme apoptosis sel kanser MCF-7 di peringkat molekular juga didapati melibatkan peningkatan protein *tumour suppressor protein* p53 tetapi tidak melibatkan transkripsi protein-protein apoptosis dari famili Bcl-2.

Di dalam eksperimen ini, modulasi dan pengaktifan semula jaringan isyarat intraselular sel yang ditunjukkan oleh ekstrak *Pandanus amaryllifolius* dan *Strobilanthes cripus* ke atas sel-sel titisan payudara merupakan strategi bagi proses perencatan proliferasi sel-sel kanser di samping menyebabkan induksi kematian sel secara apoptosis.

ACKNOWLEDGEMENT

“All our dreams can come true, if we have the courage to pursue them” -Walt Disney.

Every single step in order to complete this meaningful research has been an immense journey of patience and support from various individuals. Their continuous support and assistance offered the impetus to complete my work.

My most sincere gratitude and appreciation goes to Prof. Dr. Asmah Rahmat, the Chairman of my supervisory committee. Her ample effort and dedication had help in getting all works and thesis delivered. I wish to convey my special gratitude to my supervisory committee members consisting of Prof. Dr. Gwendoline Ee, Dr. Norjahan Banu Alitheen and Dr. Abdah Md Akim for their insight advices, guidance and support.

Deepest gratitude is expressed to University Putra Malaysia for funding this research work under university RUGS grant (vote no 91058) as well as for providing financial assistance under Graduate Research Fellowship scheme.

My special appreciation also forward to my fellow lab mates researchers and staffs from Department of Nutrition and Dietetics; Department of Cell Biology and Biomolecular and Department of Chemistry for their helpfulness and assistance. I am also indebted to Jillian and Sam for being my pillars of strength and for their wonderful companionship all these years. My warmest love and gratitude goes to my beloved parents and family

members for their endless care, love and support. For the many people who walked this journey with me, I am enormously grateful and indebted. May God bless all individuals above with constant love, health and happiness.

Hueh Zan

May, 2010



I certify that a Thesis Examination Committee has met on May 2010 to conduct the final examination of Chong Hueh Zan on her thesis entitled “Suppression effects of *Pandanus amarylfolius* and *Strobilanthes cripus* on the growth of breast cancer cells by inducing p53-mediated apoptotic pathway” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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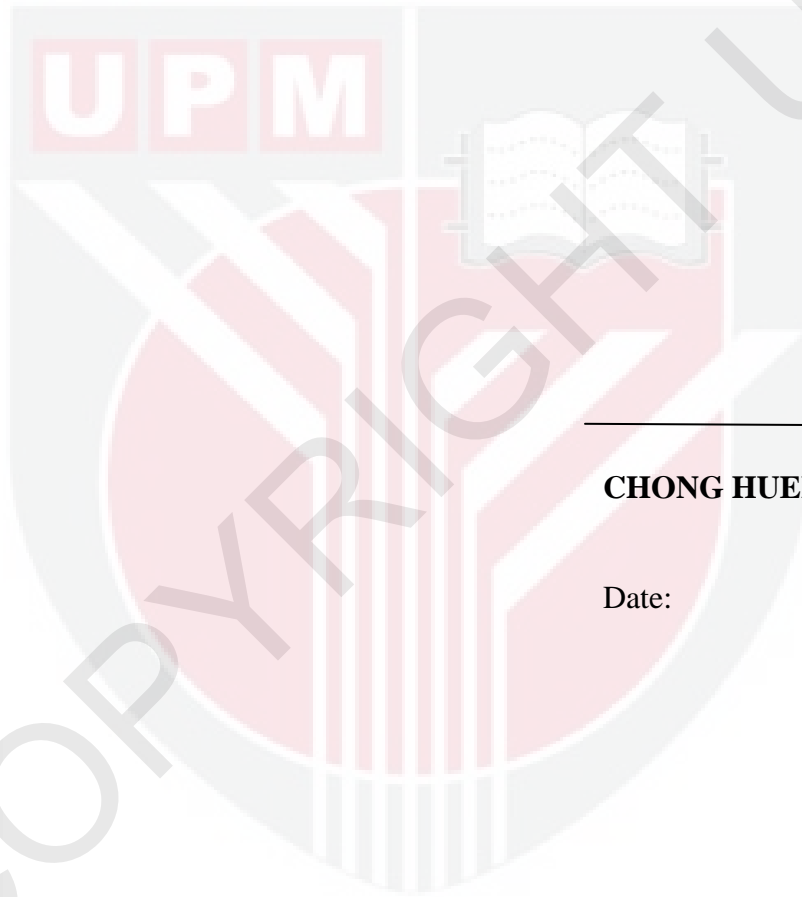
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



CHONG HUEH ZAN

Date:

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