UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF CELL CULTURES AND CYTOTOXIC ACTIVITY OF *PERESKIA BLEO* (KUNTH) DC. EXTRACTS

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OPTIMISATION OF CELL CULTURES AND CYTOTOXIC ACTIVITY OF *PERESKIA BLEO* (KUNTH) DC. EXTRACTS

By

NOOR ANILIZAWATIMA SULONG

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia in Fulfilment of the Requirement for the
Degree of Master of Science

APRIL 2011

Dedicated to my parent, husband and kids.

I love you all.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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April 2011

Chairman : Associate Professor Norihan Mohd Saleh, Ph.D.

Faculty : Biotechnology and Biomolecular Sciences

Pereskia bleo (KUNTH) DC is an edible leafy cactus which is known to have anticancer properties among the traditional medicine practitioners. However, up to now there is no scientific evidence to illustrate and confirm such claims. Thus, to discover the potential of *P.bleo* as a medicinal plants against breast cancer, further research has been conducted. Leaf tissues of *P.bleo* were used as explants for the induction of callus and cell suspension cultures on different MS media supplemented with 24 different amounts and combinations of NAA and BAP. Callus induced on MS medium supplemented with 2 mg/l NAA and 2 mg/l BAP (Media K) and on MS medium supplemented with 16 mg/l NAA and 2 mg/l BAP (Media W) showed good growth with formation of friable calli. Thus, these cultures were selected for initiation of cell suspension cultures. Elicitation of secondary metabolites was also attempted using pectin and jasmonic acid. Various extracts of *P.bleo* were prepared and assessed for their cytotoxic activities against two types of cancer cell lines (MCF-7, MDA-MB-231) and against non-tumour 3T3 mouse fibroblast cells. The chloroform crude extracts of the old leaves exhibited the strongest

cytotoxic activity against the MCF-7 cell lines with IC_{50} value of 25.0 µg/ml and slight cytotoxic activity against MDA-MB-231 cells at IC_{50} 64.0 µg/ml. The chloroform extracts of young leaves and the methanol extracts of the old leaves exhibited high cytotoxic activity against the MDA-MB-231 cells with IC_{50} = 47.0 µg/ml and 43.0 µg/ml, respectively. The non-polar extracts of cell suspension cultures were also found to exhibit cytotoxic activity against the MCF-7 cell lines tested. The petroleum ether extracts of cell suspension in Medium W was found to be most cytotoxic with IC_{50} < 17.5 µg/ml. The IC_{50} values of chloroform extracts of the cell suspension cultured in the medium W containing jasmonic acid was 27.5 µg/ml and medium W containing pectin was 54.0 µg/ml. The IC_{50} values for Semi-Purified Compound 1 and Semi-Purified Compound 2 against MCF-7 cells were 2.4 µg/ml and 10.4 µg/ml, respectively. Phytochemical analysis of the extracts using the TLC technique revealed that the extracts of the *in-vitro* cultures showed different TLC profile when compared to the extracts of the field grown plant. Except for the callus and cell suspension cultures, the leaves of *P.bleo* were found to contain large amount of alkaloids, terpenoids and saponins. Steroids and triterpenes were found in the non-polar extracts of the leaves and its cell suspension cultures. This study also demonstrated that both the leaves and the *in-vitro* cultures of *P.bleo* (KUNTH) DC. contain potent cytotoxic compounds against the MCF-7 and MDA-MB-231 cell lines. The non-cytotoxic activities of the chloroform extract of the leaves toward the non-tumour 3T3 mouse fibroblasts indicated that the non-polar crude extracts of the leaves exhibited selective mode of inhibition between tumour and non-tumour cells. The result obtained support the reputation of this species as potential anticancer plant.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN SEL KULTUR DAN AKTIVITI SITOTOKSIK EKSTRAK *PERESKIA BLEO* (KUNTH) DC.

Oleh

NOOR ANILIZAWATIMA SULONG

April 2011

Pengerusi : Profesor Madya Norihan Mohd Saleh, Ph.D.

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Pereskia bleo (KUNTH) DC ialah kaktus berdaun yang boleh dimakan yang digunakan oleh pengamal perubatan untuk mengubati kanser payudara. Walaubagaimanapun, sehingga kini, tiada bukti saintifik untuk menjelaskan dan mengesahkan dakwaan itu. Oleh itu, untuk mengenalpasti potensi *P.bleo* sebagai pokok perubatan untuk merawat kanser payudara, kajian mendalam telah dijalankan. Tisu daun *P.bleo* digunakan sebagai eksplan untuk mengaruh kalus dan kultur sel ampaian di dalam 24 jenis media MS yang berbeza ditambah dengan pelbagai kepekatan dan kombinasi NAA dan BAP. Kalus yang diaruh di atas media MS beserta 2 mg/l NAA dan 2 mg/l BAP (Media K) dan di atas media MS beserta 16 mg/l NAA dan 2 mg/l BAP (Media W) menunjukkan pertumbuhan bagus dengan penghasilan kalus mudah terlerai. Oleh itu, kultur ini telah dipilih untuk mengasaskan kultur sel ampaian. Percubaan meningkatkan jumlah metabolit sekunder juga dibuat menggunakan pektin dan asid jasmonik. Pelbagai ekstrak dari *P.bleo* telah disediakan dan aktiviti sitotoksik mereka terhadap dua jenis sel kanser

(MCF-7, MDA-MB-231) dan sel normal fibroblast tikus 3T3 telah dikaji. Ekstrak klorofom daun tua menunjukkan aktiviti sitotoksik yang terkuat terhadap sel MCF-7 dengan nilai IC_{50} sebanyak 25.0 µg/ml dan sedikit aktiviti sitotoksik terhadap sel MDA-MB-231 dengan IC_{50} sebanyak 64.0 µg/ml. Ekstrak klorofom daun muda dan ekstrak metanol daun tua menunjukkan aktiviti sitotoksik yang tertinggi terhadap sel MDA-MD-231, dengan nilai IC_5 masing-masing adalah 47.0 µg/ml dan 43.0 µg/ml. Ekstrak tidak-polar kultur sel ampaian juga menunjukkan aktiviti sitotoksik terhadap sel MCF-7. Ekstrak petroleum eter sel ampaian di dalam Media W didapati paling sitotoksik dengan IC_{50}< 17.5 µg/ml. Nilai IC_{50} bagi ekstrak klorofom sel ampaian yang dikultur dalam media W yang ditambah asid jasmonik adalah 27.5 µg/ml dan media W yang ditambah pektin adalah 54.0 µg/ml. Nilai IC_{50} bagi Sebatian Separa-tulen 1 dan Sebatian Separa-tulen 2 terhadap sel MCF-7 masing-masing adalah 2.4 µg/ml dan 10.4 µg/ml. Analisis fitokimia terhadap ekstrak-ekstrak ini menggunakan teknik TLC menunjukkan bahawa ekstrak dari kultur *in-vitro* menunjukkan TLC profil yang berbeza berbanding ekstrak dari pokok semulajadi. Kecuali pada kalus dan sel ampaian daun, daun *P.bleo* didapati mengandungi alkaloid, terpenoid dan saponin yang banyak. Steroid dan triterpena dijumpai di dalam ekstrak tidak-polar daun dan sel ampaian. Kajian ini juga menunjukkan bahawa kedua-dua daun dan kultur *in-vitro P.bleo* (KUNTH) DC. mengandungi sebatian sitotoksik yang berkesan terhadap sel MCF-7 dan MDA-MB-231. Aktiviti tidak-sitotoksik ekstrak klorofom daun terhadap sel non-tumor 3T3 tikus fibroblast menunjukkan bahawa ekstrak mentah tidak-polar daun mempunyai mod penyekatan selektif di antara sel tumor dan non-tumor. Keputusan yang diperolehi menyokong reputasi spesis ini sebagai tumbuhan potensi antikanser.

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I certify that a Thesis Examination Committee has met on 18th April 2011 to conduct the final examination of Noor Anilizawatima bt Sulong on her thesis entitled "Optimisation of Cell Cultures and Cytotoxic Activity of *Pereskia bleo* (Kunth) DC. Extracts" 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 Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the supervisory committee were as follows:

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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 other institutions.

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NOOR ANILIZAWATIMA BT SULONG

Date : 18 April 2011

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