UNIVERSITI PUTRA MALAYSIA

SHOOT MULTIPLICATION, MICROTUBER PRODUCTION AND EVALUATION OF ANTIOXIDANT AND CANCER CELL CYTOTOXICITY ACTIVITIES OF CHLOROPHYTUM SANT. & FERNANDEZ

MEHDI FARSHAD ASHRAF

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By

MEHDI FARSHAD ASHRAF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2012

Dedicated

To my beloved wife, Elmira for all her love, support and patience and my son Arshan. Special thanks to my father and mother, who inspired me

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the Degree of Doctor of Philosophy

SHOOT MULTIPLICATION, MICROTUBER PRODUCTION AND EVALUATION OF ANTIOXIDANT AND CANCER CELL CYTOTOXICITY ACTIVITIES OF CHLOROPHYTUM SANT. & FERNANDEZ

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Chair: Associate Professor Maheran Bt Abd Aziz, PhD

Faculty: Agriculture

Chlorophytum borivilianum is an important medicinal plant. The tuberous roots possess immunomodulatory and adaptogenic properties, and are used to cure impotency, sterility and enhance male potency. The seeds have poor germination percentage (11-24%), low viability and long dormancy period. Considering Safed musli is an endangered species and the limited availability of planting materials, the use of tissue culture system could provide a rapid method for mass propagating the plant. In this study, young shoot buds of *C. borivilianum* were cultured on Murashige and Skoog (MS) medium containing BAP and Kn, both at 0, 8.88, 17.8 and 26.6 µM, either individually or in combination. Proliferated shoots were subcultured on fresh medium of the same constituents at 3 weeks interval. The combination of 8.88 µM BAP and 8.88 µM Kn was most suitable for shoot multiplication and elongation of *C. borivilianum*. *In vitro* shoot tips of *C. borivilianum* were cultured for microtuber induction on MS solid medium containing different concentrations of 0, 315, 630, 950, 1265 and 1580 µM CCC combined with

30, 60 and 90 g l^{-1} of sucrose. The combination of 950 µM CCC and 60 g l^{-1} sucrose produced high number of microtubers with increased length. Upon using stationary liquid MS medium containing the different combinations of CCC and sucrose as applied for solid medium, microtuber production was improved and the best combination was also in medium containing 950 µM CCC and 60 g l^{-1} sucrose. For optimization of microtuber production, comparison between solid, stationary and shake liquid cultures was carried out. Liquid culture with shaking at 80rpm resulted in more than 2.5 fold increase in microtuber production compared to solid culture. The study was extended using RITA system for scaling up of microtuberization. Microtuberization was enhanced and hyperhydricity was eliminated using 15 min immersion time for every 60 min rest period. Substitution of the optimized liquid microtuberization medium (OLMM) containing 950 µM CCC and 60g l^{-1} sucrose with liquid hormone-free MS medium (MSO) on week 6 of culture was more economical for microtuberization than maintaining the culture throughout the 9 weeks on OLMM. Quantitation of total saponin showed a higher content in microtubers than in mother plant tubers. Analysis of antioxidant activity of crude and total saponin extracts from mother plant tubers of *C. borivilianum* indicated higher antioxidant activity of crude extract using DPPH and BCB assays, while higher chelating activity was shown by total saponin using FIC assay. Cytotoxicity evaluation of crude and total saponin extracts against MCF7, PC3 and HCT116 cancer cell lines using MTT cell viability assay indicated a higher cytotoxicity activity of the crude extract than the total saponin fraction on all cell lines, being most effective and selective on MCF7 human breast cancer cell line. In conclusion, shoot

regeneration on solid medium, microtuber production on solid medium, in liquid medium and upscaling of microtuber production using a RITA system had been successfully established for *C. borivilianum.* The study also provided valuable information on the antioxidant and cytotoxicity activities of crude and total saponin extracts from tubers of *C. borivilianum.*

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

PENGGANDAAN PUCUK, PENGELUARAN MIKROTUBER, DAN PENILAIAN ANTIOKSIDAN DAN AKTIVITI SITOTOKSIK SEL KANSER BAGI CHLOROPHYTUM BORIVILIANUM SANT & FERNANDEZ

Oleh:

MEHDI FARSHAD ASHRAF

Julai 2012

Pengerusi: Profesor Madya Maheran bt Abd Aziz, PhD

Fakulti: Pertanian

Chlorophytum borivilianum adalah tumbuhan ubatan penting. Akar tuber *C. borivilianum* mempunyai sifat immunomodulatori dan adaptogenik, dan digunakan untuk menyembuhkan masalah mati pucuk, kemandulan dan meningkatkan potensi lelaki. Biji benih tanaman ini mempunyai peratusan percambahan rendah (11-24%), kurang kecergasan dan tempoh dormansi yang panjang. Oleh kerana Safed musli merupakan spesies yang terancam dan ketersediaan bahan tanaman adalah terhad, sistem kultur tisu boleh digunakan sebagai kaedah yang cepat untuk pembiakan tanaman ini. Dalam kajian ini, tunas pucuk muda *C. borivilianum* telah dikultur pada medium Murashige dan Skoog (MS) yang mengandungi hormon BAP dan Kn, kedua-dua hormon ini pada kepekatan 0, 8.88, 17.8 dan 26.6 µM, secara individu atau kombinasi. Pucuk yang terhasil disubkultur pada medium baru dengan kandungan yang sama setiap 3 minggu. Gabungan 8.88 µM BAP dan 8.88

μm Kn didapati paling sesuai untuk penggandaan dan pemanjangan pucuk *C. borivilianum*. Pucuk *in vitro C. borivilianum* telah dikulturkan untuk induksi mikrotuber pada medium MS pepejal yang mengandungi kepekatan CCC berbeza 0, 315, 630, 950, 1265 dan 1580 μM digabungkan dengan 30, 60 dan 90 g l^{-1} sukrosa. Gabungan 950 μM CCC dan 60 g l^{-1} sukrosa menghasilkan bilangan mikrotuber yang tinggi dan lebih panjang. Dengan penggunaan medium MS cecair statik yang mengandungi kombinasi berbeza CCC dan sukrosa sama seperti yang digunakan untuk medium pepejal, pengeluaran mikrotuber bertambah baik dan kombinasi terbaik juga adalah pada medium mengandungi 950 μM CCC dan 60 g l^{-1} sukrosa. Untuk mengoptimumkan penghasilah mikrotuber, perbandingan di antara kultur pepejal, cecair statik dan cecair goncang telah dijalankan. Kultur cecair dengan penggoncangan pada 80rpm menghasilkan lebih 2.5 kali ganda pembentukan mikrotuber berbanding kultur pepejal. Kajian diteruskan menggunakan sistem RITA untuk meningkatkan penghasilan mikrotuber. Pengeluaran mikrotuber dapat dipertingkatkan dan hiperhidrisiti dihapuskan dengan kaedah rendaman selama 15 minit bagi setiap 60 minit masa rihat. Pengukuran jumlah saponin menunjukkan kandungannya lebih tinggi di dalam mikrotuber berbanding tuber induk. Analisis aktiviti antioksidan bagi ekstrak mentah dan jumlah saponin dari tuber induk *C. borivilianum* menunjukkan aktiviti antioksidan yang lebih tinggi bagi ekstrak mentah menggunakan asai DPPH dan BCB, sementara aktiviti pengkelat lebih tinggi ditunjukkan oleh jumlah saponin melalui asai FIC. Penilaian ke atas aktiviti sitotoksik bagi ekstrak mentah dan jumlah saponin terhadap sel kanser MCF7, PC3 dan HCT116 menggunakan asai MTT menunjukkan aktiviti

sitotoksik yang lebih tinggi bagi ekstrak mentah berbanding jumlah saponin terhadap semua sel kanser, dan yang paling berkesan dan selektif adalah terhadap sel kanser payudara manusia MCF7. Rumusannya, regenerasi pucuk di atas medium pepejal, pengeluaran mikrotuber di atas medium pepejal, di dalam medium cecair dan peningkatan pengeluaran mikrotuber menggunakan sistem RITA telah berjaya dibangunkan untuk *C. borivilianum.* Kajian ini juga memberikan maklumat berguna mengenai aktiviti antioksidan dan sitotoksik bagi ekstrak mentah dan jumlah saponin dari tuber *C. borivilianum.*

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I certify that a Thesis Examination Committee has met on 23rd July 2012 to conduct the final examination of Mehdi Farshad Ashraf on his thesis entitled "SHOOT MULTIPLICATION, MICROTUBER PRODUCTION AND EVALUATION OF ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF *CHLOROPHYTUM BORIVILIANUM*" in accordance with the Universities and University Collages 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.

Members of the Thesis Examination Committee were as followed:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment for the degree of Doctor of Philosophy. The members of 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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MEHDI FARSHAD ASHRAF

Date:

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