



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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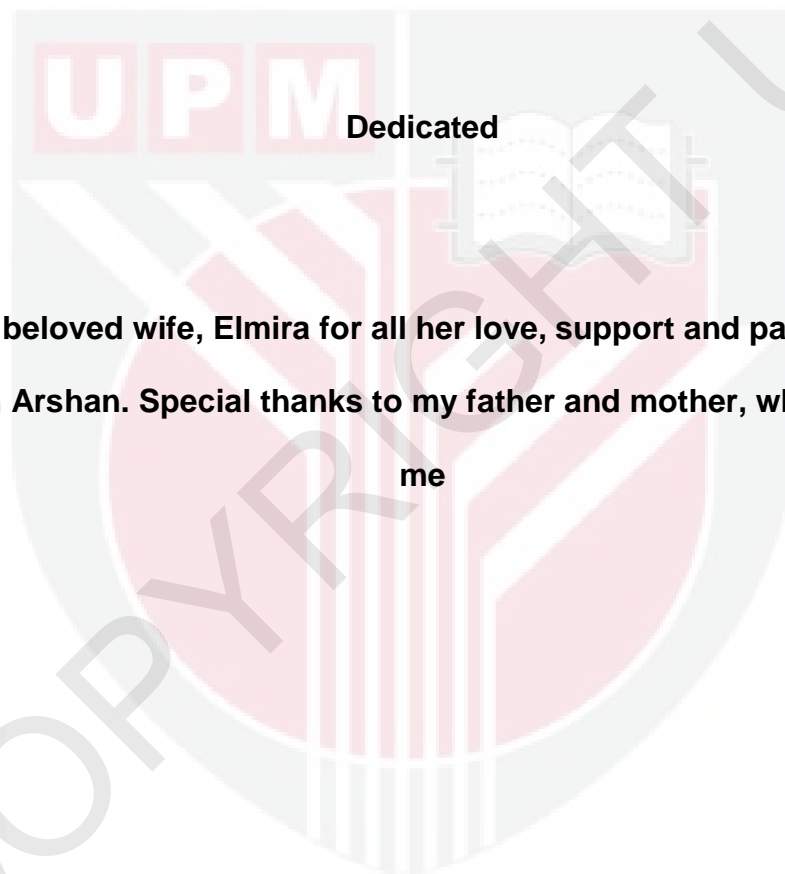


By

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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July 2012

Chair: Associate Professor Maheran Bt Abd Aziz, PhD

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Chlorophytum borivillianum 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. borivillianum* 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. borivillianum*. *In vitro* shoot tips of *C. borivillianum* 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**

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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

$\mu\text{m Kn}$ 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 penghasilan 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 rehat. 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.

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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.



MEHDI FARSHAD ASHRAF

Date:



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xx

CHAPTER

1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Botany	5
2.2 Propagation of <i>C. borivilianum</i>	7
2.2.1 Organogenesis	7
2.2.2 <i>In vitro</i> tuber formation	10
2.2.3 Benefits of liquid culture	14
2.2.4 Advantages of bioreactor and temporary immersion system for plant micropropagation	16
2.3 Phytochemistry and pharmacognocny	20
3 SHOOT MULTIPLICATION AND MICROTUBER FORMATION USING SOLID CULTURE	24
3.1 Introduction	24
3.2 Materials and Methods	26
3.2.1 Explant preparation and culture	26
3.2.1.1 Shoot multiplication and elongation	26
3.2.1.2 Microtuberization	27
3.2.2 Treatments	27
3.2.2.1 Shoot multiplication and elongation	27
3.2.2.2 Microtuberization	28
3.2.3 Experimental design and data analysis	29
3.3 Results and Discussion	29
3.3.1 Shoot multiplication and elongation	29
3.3.1.1 Effect of BAP on shoot multiplication and elongation	29
3.3.1.2 Effect of Kn on shoot multiplication and elongation	30
3.3.1.3 Interaction between BAP and Kn on shoot multiplication and elongation	32
3.3.2 Microtuberization	36
3.3.2.1 Effect of CCC on microtuberization	36

3.3.2.2	Effect of sucrose on microtuberization	37
3.3.2.3	Interaction effect of CCC and sucrose on microtubereization	40
3.4	Conclusion	43
4	APPLICATION OF LIQUID CULTURE AND TEMPORARY IMMERSION SYSTEM ON MICROTUBERIZATION	45
4.1	Introduction	45
4.2	Materials and Methods	47
4.2.1	Explant preparation and culture	47
4.2.1.1	Microtuberization using stationary liquid culture	47
4.2.1.2	Comparison of solid, stationary liquid and shake liquid cultures on microtuberization	50
4.2.1.3	Effect of different immersion time and medium substitution in RITA [®] system on microtuberization	51
4.2.2	Treatments	51
4.2.2.1	Microtuberization using stationary liquid culture	51
4.2.2.2	Comparison of solid, stationary liquid culture and shake liquid cultures on microtuberization	52
4.2.2.3	Effect of immersion time frequency and medium substitution in RITA [®] system on microtuber formation of <i>C. borivillianum</i>	53
4.2.3	Description of the automated RITA [®]	55
4.2.4	Data analysis	57
4.3	Results and Discussion	57
4.3.1	Microtuberization using stationary liquid culture in <i>C. borivillianum</i>	57
4.3.1.1	Effect of CCC on microtuberization	57
4.3.1.2	Effect of Sucrose on microtuberization	58
4.3.1.3	Interaction of CCC and sucrose on microtuberization	60
4.3.2	Comparison of culture systems on microtuberization in <i>C. borivillianum</i>	69
4.3.2.1	Effect of culture system on mean number and length of microtubers	69
4.3.2.2	Effect of culture system on mean growth index and hyperhydricity	72
4.3.3	Effect of immersion time frequency and medium substitution in RITA [®] on microtuber formation of <i>C. borivillianum</i>	76
4.3.3.1	Effect of immersion time frequency on microtuber formation	76
4.3.3.2	Effect of medium substitution on microtuber formation	81
4.4	Conclusion	88

5	PHYTOCHEMICAL ANALYSIS OF <i>C. BORIVILIANUM</i>	89
5.1	Introduction	89
5.2	Materials and Methods	90
5.2.1	Plant Material	90
5.2.2	Chemicals	90
5.2.3	Extraction of crude extract from tubers of <i>C. borivilianum</i>	91
5.2.4	Determination and quantitation of total saponin in mother plant tubers and <i>in vitro</i> tubers	92
5.2.4.1	Preparation of total saponin extract	92
5.2.4.2	Preparation of material for total saponin determination	95
5.2.4.3	Calibration curve	95
5.2.4.4	Quantitation of total saponins	96
5.2.5	Methods for screening antioxidant activity	96
5.2.5.1	DPPH radical scavenging assay	96
5.2.5.2	Ferrous ion chelating (FIC) assay	98
5.2.5.3	β -carotene bleaching (BCB) assay	100
5.3	Results and Discussion	102
5.3.1	Quantitation of total saponin in mother plant tubers and <i>in vitro</i> tubers	102
5.3.2	Screening of antioxidant activity	106
5.3.2.1	DPPH radical scavenging activity	106
5.3.2.2	Ferrous ion chelating (FIC) activity	108
5.3.2.3	β -carotene bleaching (BCB) activity	109
5.4	Conclusion	113
6	CYTOTOXIC ACTIVITY SCREENING ON <i>C. BORIVILIANUM</i>	114
6.1	Introduction	114
6.2	Materials and Method	115
6.2.1	Plant material and extract preparation for screening	115
6.2.2	Cultures, chemicals and equipments	115
6.2.3	Instruments	116
6.2.4	Preparation of extracts	117
6.2.5	Cell culture	119
6.2.6	MTT Assay	120
6.3	Results and Discussion	122
6.3.1	Growth inhibition values (GI_{50} , TGI and LC_{50}) of <i>C. borivilianum</i> total saponin extracts in MCF-7, PC3 and HCT 116 cell lines	122
6.3.2	Morphological Changes on MCF7 treated cells	126
6.4	Conclusion	129
7	SUMMARY, CONCLUSION AND FUTURE DIRECTION	130
7.1	Discussion	130
7.2	Conclusion	132
7.3	Future direction	133
	REFERENCES	134

APPENDICES

156

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164



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