



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES AND *IN VITRO*
CANCER CHEMOPREVENTIVE EFFECTS OF *AVERRHOA BILIMBI* L.
EXTRACT ON HUMAN BREAST AND CERVICAL ADENOCARCINOMA**

YAN SEE WAN

FPSK(p) 2012 15

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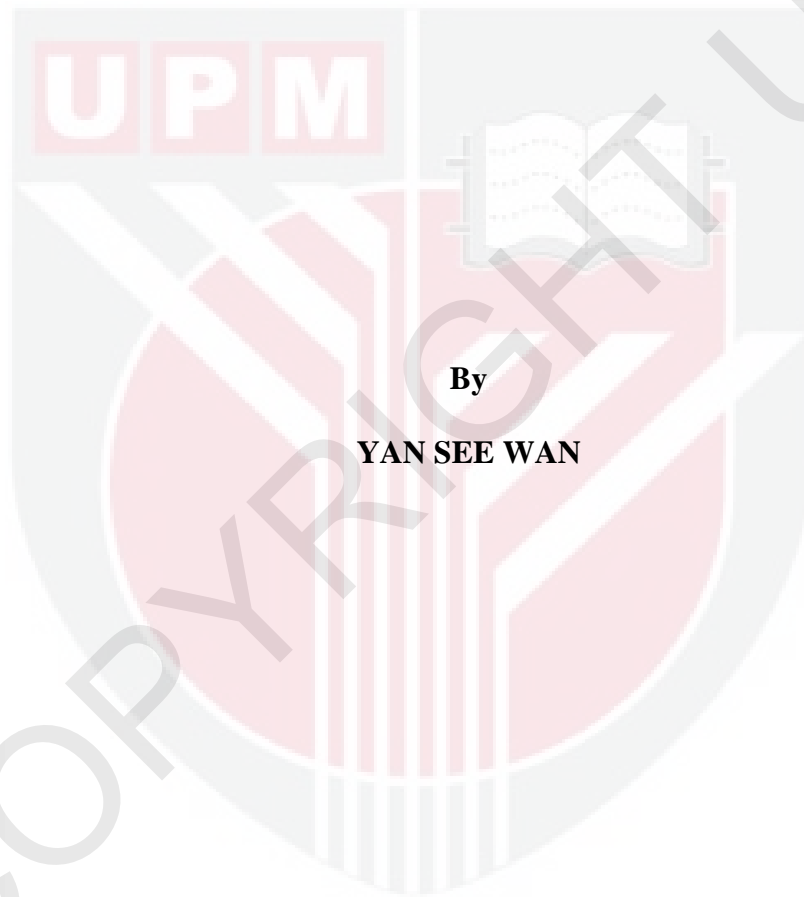
The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. It features a red and white color scheme. At the top left, the letters 'UPM' are written in white on a red background. In the center, there is a stylized white figure that resembles a person or a tree, set against a red background. To the right of this figure is an open book with white pages. The entire emblem is surrounded by a white border.

YAN SEE WAN

**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2012

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EXTRACT ON HUMAN BREAST AND CERVICAL ADENOCARCINOMA**



By

YAN SEE WAN

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

June 2012

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

ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES AND *IN VITRO* CANCER CHEMOPREVENTIVE EFFECTS OF *AVERRHOA BILIMBI* L. EXTRACT ON HUMAN BREAST AND CERVICAL ADENOCARCINOMA

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June 2012

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High consumption of fruits has been associated with a reduced risk of various degenerative diseases such as cancer. These protective properties are reckoned to be linked to the micronutrients and non-nutritive phytochemicals present in fruits. This study was conducted to investigate the nutritional compositions, total phenolic and flavonoid contents, antioxidant capacity, antioxidant vitamins, phenolic acid and flavonoid compounds, as well as anticancer potential of two types of fruits endemic to Malaysia: *Averrhoa bilimbi* (bilimbi) and *Averrhoa carambola* (carambola). Commonly consumed among the locals, these underutilized fruits have been popularly practiced as folk medicine since ancient times.

Results from nutritional compositions analyses showed that bilimbi possessed higher fat and total dietary fibre but comparable moisture, ash, carbohydrate and protein content with that of carambola. Results from Folin-Ciocalteu, aluminium chloride colourimetric assays and antioxidant vitamins analyses indicated a higher antioxidant vitamins and flavonoid content in bilimbi while carambola with higher total phenolic. β -carotene bleaching and DPPH radical scavenging assays suggested that carambola displayed stronger antioxidant capacity and was positively correlated with its total phenolic content.

The presence of non-nutritive phytochemicals in both fruits was evaluated by HPLC analysis based on the optimized extraction conditions performed through single-factor experiments. Analysis revealed that optimal extraction conditions were 80% ethanol at 65 °C for 8 hours in both fruit extracts. Phenolic compounds extracted by the optimal extraction conditions were subjected to HPLC analysis. Epigallocatechin and catechin were identified as the key flavonoids in bilimbi and carambola, respectively while sinapic acid and syringic acid were the predominant phenolic acid in bilimbi and carambola, respectively.

Cytotoxic and anticancer potential of bilimbi and carambola ethanolic extracts were examined on various human cancer cell lines (breast, cervical, colon, liver and ovarian adenocarcinoma) as well as non-malignant Chang Liver cell line. Findings from MTS assay implied a 50% growth inhibitory effect induced by bilimbi extract on cervical cancer cell line (HeLa) and non-hormone dependent breast cancer cell line (MDA-MB-

231) at 70 and 90 µg/ml, respectively without any cytotoxic effect on non-malignant Chang Liver cell line. In contrast, carambola extract was unable to exert any growth inhibitory effect even concentration was increased up to 200 µg/ml. Further investigations to determine apoptogenic potential of bilimbi extract revealed typical morphological features of apoptosis as well as formation of ladder-like pattern in bilimbi-treated HeLa and MDA-MB-231. Apoptosis and antiproliferative effects were further evidenced with the increased in sub G1 population and accumulation of cells with a 2N DNA content in cell cycle analysis, suggesting perturbation at G0/G1 checkpoint. BrdU incorporation cell proliferation assay indicated a decreased in cells synthesis (S phase). Released of cytochrome c coupled with upregulation of caspase-3/7 and caspase-9 expression indicated that molecular mechanism in the induction of apoptosis by bilimbi on HeLa and MDA-MB-231 was through mitochondria-mediated intrinsic pathway, which also involve upregulation of pro-apoptotic Bax protein and downregulation of anti-apoptotic Bcl-2 protein. Upregulation of tumour suppressor p53 protein was detected in HeLa upon exposure to bilimbi, as opposed to MDA-MB-231 which was found to be independent of p53. Hence, bilimbi induced apoptosis through a p53-dependent mitochondrial pathway in HeLa and a p53-independent mitochondrial pathway in MDA-MB-231.

Based on the antioxidant and phytochemicals properties as well as anticancer potential, bilimbi offers a promising candidate for cancer chemoprevention strategy. As a natural product, bilimbi is potentially safe and beneficial for pharmaceutical and health industries worth further research and investigations.

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

**KANDUNGAN ANTIOXIDAN DAN FITOKIMIA DAN KESAN *IN VITRO*
KEMO PENCEGAHAN KANSER EKSTRAK *AVERRHOA BILIMBI L.* PADA
ADENOKARSINOMA PAYUDARA DAN SERVIK MANUSIA**

Oleh

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Jun 2012

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Kekerapan pengambilan buah-buahan berkait rapat dengan pengurangan risiko penyakit degeneratif seperti kanser. Ciri-ciri perlindungan ini adalah berkaitan dengan mikronutrien dan fitokimia yang terdapat dalam buah-buahan. Kajian ini dijalankan untuk menyiasat komposisi pemakanan, jumlah kandungan fenolik dan flavonoid, kapasiti antioksidan, vitamin antioksidan, sebatian asid fenolik dan flavonoid, serta potensi anti kanser dua jenis buah-buahan yang endemik kepada Malaysia: *Averrhoa bilimbi* (bilimbi) dan *Averrhoa carambola* (carambola). Biasanya digunakan di kalangan penduduk tempatan, buah-buahan terpinggir ini kerap diamalkan sebagai ubat tradisional sejak zaman purba.

Keputusan analisis komposisi pemakanan menunjukkan bahawa bilimbi memiliki lemak dan serat yang tinggi tetapi kandungan air, abu, karbohidrat dan protein yang standing dengan carambola. Keputusan kaedah Folin-Ciocalteu, ujian kolorimetrik aluminium klorida dan vitamin antioksidan analisis menunjukkan bahawa bilimbi mengandungi vitamin antioksidan dan flavonoid yang lebih tinggi manakala carambola mengandungi jumlah fenolik yang lebih tinggi. Ujian pelunturan β -karotena dan pemerangkapan radikal DPPH mencadangkan bahawa carambola memaparkan kapasiti antioksidan yang kuat dan berkait positif dengan jumlah kandungan fenolik.

Kehadiran fitokimia dalam kedua-dua buah-buahan dinilai oleh analisis HPLC berdasarkan keadaan pengekstrakan optima yang ditentukan melalui uji kaji satu faktor. Analisis menunjukkan bahawa keadaan pengekstrakan optima adalah 80% etanol pada 65 °C selama 8 jam pada kedua-dua ekstrak buah-buahan. Sebatian fenolik kemudian diekstrak dalam keadaan pengekstrakan optima melalui analisis HPLC. Epigallocatechin dan catechin masing-masing dikenal pasti sebagai flavonoid utama dalam bilimbi dan carambola manakala asid sinapic dan asid syringic masing-masing dikenal pasti sebagai asid fenolik utama dalam bilimbi dan carambola.

Potensi sitotoksik dan anti kanser ekstrak etanol bilimbi dan carambola disaring pada beberapa sel kanser manusia (adenokarsinoma payudara, serviks, kolon, hati dan ovari) serta sel normal Chang Liver. Keputusan analisis MTS menunjukkan bahawa ekstrak bilimbi merencat pertumbuhan sel kanser serviks (HeLa) dan payudara (MDA-MB-231) sebanyak 50% masing-masing dengan nilai 70 dan 90 $\mu\text{g/ml}$ tanpa kesan sitotoksik ke

atas sel normal Chang Liver. Sebaliknya, ekstrak carambola tidak mampu merencat pertumbuhan sebarang sel kanser walaupun kepekatan ekstrak ditingkatkan sehingga 200 µg/ml. Siasatan lanjut untuk menentukan potensi apoptosis ekstrak bilimbi menunjukkan bahawa kesan ekstrak bilimbi ke atas HeLa dan MDA-MB-231 menunjukkan ciri-ciri morfologi lazim apoptosis serta pembentukan pola DNA ladder. Kesan apoptosis dan anti proliferasi juga dibuktikan melalui peningkatan fasa sub G1 dan peningkatan sel dengan kandungan 2N DNA dalam analisis kitaran sel yang menyebabkan pembantutan proses kitaran sel di fasa G0/G1. Ujian sel proliferasi BrdU menunjukkan pengurangan sintesis sel (fasa S). Pengaktifan sitokrom c serta peningkatan caspase-3/7 dan caspase-9 menunjukkan bahawa mekanisme induksi apoptosis oleh bilimbi pada HeLa dan MDA-MB-231 di peringkat molekul adalah melalui laluan intrinsik mitokondria yang juga melibatkan peningkatan protein pro apoptosis Bax dan penurunan protein anti apoptosis Bcl-2. Pendedahan HeLa kepada ekstrak bilimbi menyebabkan peningkatan protein *tumour suppressor* p53, sebaliknya, induksi apoptosis oleh bilimbi pada MDA-MB-231 didapati tidak melibatkan protein *tumour suppressor* p53. Oleh itu, induksi apoptosis oleh bilimbi pada HeLa adalah bergantung kepada p53 dan melalui laluan intrinsik mitokondria manakala pada MDA-MB-231 adalah tidak bergantung kepada p53 dan melalui laluan intrinsik mitokondria.

Berdasarkan ciri-ciri antioksidan dan fitokimia serta potensi anti kanser, bilimbi berupaya digunakan dalam strategi kemo pencegahan kanser. Sebagai produk semulajadi, bilimbi adalah selamat dan bermanfaat untuk industri farmaseutikal dan kesihatan serta amat sesuai untuk penyelidikan dan siasatan lanjut.

ACKNOWLEDGEMENTS

"Success is not final, failure is not fatal; it is the courage to continue that counts"

~ Winston Churchill ~

Journey through the completion of this research project would not have been possible without the generous advice, encouragement and assistance of many people along the way. Their continuous support and contribution has provided me the desire to pursue my dream and the courage to make a difference in life.

My utmost gratitude and heartiest appreciation goes to the chairman of my supervisory committee, Prof. Dr. Asmah Rahmat who attracted me with interest to the world of scientific research. Her valuable guidance and input has helped in the accomplishment of this project. I would also like to acknowledge my supervisory committees, Dr. Rajesh Ramasamy and Assoc. Prof. Dr. Noorjahan Banu Mohamed Alitheen who gave thoughtful insight in their area of expertise. Thanks for all the opportunities and painstaking efforts throughout this journey.

Sincere gratitude is extended to my fellow laboratory mates who are willing to share their knowledge and opinions during experiments. I am deeply indebted to them for being my pillars of strength and amazing companions through thick and thin. I realize I am not alone through this journey. Not forgetting laboratory staffs from Department of Nutrition and Dietetics, Department of Pathology and Department of Cell and Molecular

Biology for their sincere help and kind assistance. Thanks for the endless cooperation and remarkable services rendered.

My warmest love and blessing goes to my supportive parents and family members for their never-ending care and comfort. Their sacrifices and unwavering support shall never be forgotten and always be appreciated. Heartfelt thanks to everyone who walk this journey with me, I am truly grateful and absolutely overwhelmed with gratitude. By God's grace may everyone be blessed with lifelong happiness, peace and joy. Thanks a million.

I certify that a Thesis Examination Committee has met on 11 June 2012 to conduct the final examination of Yan See Wan on her thesis entitled “Antioxidant and Phytochemical Properties and *In Vitro* Cancer Chemopreventive Effects of *Averrhoa bilimbi* L. Extract on Human Breast and Cervical Adenocarcinoma” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the students be awarded the degree of 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 at any other institution.



YAN SEE WAN

Date: 11 June 2012

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxv
CHAPTER	
1 INTRODUCTION	1
1.1 Significance of Study	4
1.2 General Objective	5
1.3 Specific Objectives	5
2 LITERATURE REVIEW	7
2.1 Cancer	7
2.1.1 Mechanism of Cancer Development	8
2.1.2 Cancer Statistic	12
2.2 Breast Cancer	14
2.2.1 Type of Breast Cancer	15
2.2.2 Risk Factors	16
2.2.3 Symptoms	20
2.2.4 Screening and Early Detection	21
2.2.5 Treatments	22
2.3 Cervical Cancer	24
2.3.1 Risk Factors	25
2.3.2 Symptoms	27
2.3.3 Screening and Early Detection	28
2.3.4 Treatments	29
2.4 Cell Death	30
2.4.1 Morphological Features of Apoptosis	31
2.4.2 Apoptosis in Cancer	33
2.5 Plant Product as Medicine	36
2.5.1 Cancer Chemoprevention	37
2.5.2 Antioxidant and Cancer	40
2.5.3 Dietary Phytochemicals and Cancer	42
2.6 Bilimbi	44
2.6.1 Food Uses	45
2.6.2 Medicinal Values	46
2.7 Carambola	48

2.7.1	Food Uses	49
2.7.2	Medicinal Values	50
3	PROXIMATE COMPOSITION AND ANTIOXIDANT CAPACITIES OF BILIMBI (<i>AVERRHOA BILIMBI</i>) AND CARAMBOLA (<i>AVERRHOA CARAMBOLA</i>)	52
3.1	Introduction	52
3.2	Materials and Methods	55
3.2.1	Materials	55
3.2.2	Sample Preparation	55
3.2.3	Proximate Analyses	56
3.2.4	Extraction	56
3.2.5	Total Phenolic Content	57
3.2.6	Total Flavonoid	57
3.2.7	Antioxidant Capacity	58
3.2.8	Antioxidant Vitamins	60
3.2.9	Statistical Analysis	62
3.3	Results	64
3.3.1	Nutritional Composition	64
3.3.2	Total Phenolic Content	66
3.3.3	Total Flavonoid	66
3.3.4	Antioxidant Capacity	67
3.3.5	Antioxidant Vitamins	68
3.3.6	Correlation	69
3.4	Discussion	74
3.5	Conclusion	80
4	PHYTOCHEMICAL PROPERTIES OF BILIMBI (<i>AVERRHOA BILIMBI</i>) AND CARAMBOLA (<i>AVERRHOA CARAMBOLA</i>)	81
4.1	Introduction	81
4.2	Materials and Methods	84
4.2.1	Materials	84
4.2.2	Sample Preparation	84
4.2.3	Extraction	85
4.2.4	Experimental Design	85
4.2.5	Total Phenolic Content	86
4.2.6	Total Flavonoid	87
4.2.7	Identification of Phenolic Compounds	87
4.2.8	Statistical Analysis	89
4.3	Results	91
4.3.1	Extraction Solvent	91
4.3.2	Extraction Temperature	94
4.3.3	Extraction Time	97
4.3.4	Phenolic Compounds Analysis	100

4.4	Discussion	103
4.5	Conclusion	105
5	ANTICANCER PROPERTIES OF BILIMBI (<i>AVERRHOA BILIMBI</i>) EXTRACT ON HUMAN CERVICAL ADENOCARCINOMA	106
5.1	Introduction	106
5.2	Materials and Methods	110
5.2.1	Materials	110
5.2.2	Sample Preparation	110
5.2.3	Extraction	112
5.2.4	Cell Cultures	112
5.2.5	MTS Assay	113
5.2.6	Morphological Observation	114
5.2.7	Acridine Orange/Propidium Iodide Double Staining	114
5.2.8	DNA Ladder Assay	115
5.2.9	TUNEL Assay	116
5.2.10	Cell Cycle Analysis	117
5.2.11	5-Bromo-2'-deoxyuridine (BrdU) Incorporation Cell Proliferation Assay	119
5.2.12	Annexin V/Propidium Iodide (PI) Staining	120
5.2.13	Caspase-8 and -9 Assay	121
5.2.14	Caspase-3/7 Assay	122
5.2.15	Cytochrome C Assay	123
5.2.16	p53, Bax and Bcl-2 Proteins Expression	124
5.2.17	Statistical Analysis	125
5.3	Results	126
5.3.1	MTS Assay	126
5.3.2	Morphological Observation	129
5.3.3	Acridine Orange/Propidium Iodide Double Staining	132
5.3.4	DNA Ladder Assay	134
5.3.5	TUNEL Assay	136
5.3.6	Cell Cycle Analysis	138
5.3.7	5-Bromo-2'-deoxyuridine (BrdU) Incorporation Cell Proliferation Assay	146
5.3.8	Annexin V/Propidium Iodide (PI) Staining	148
5.3.9	Caspase-8 and -9 Assay	153
5.3.10	Caspase-3/7 Assay	156
5.3.11	Cytochrome C Assay	158
5.3.12	p53, Bax and Bcl-2 Proteins Expression	160
5.4	Discussion	165
5.5	Conclusion	174

6	ANTICANCER PROPERTIES OF ON BILIMBI (<i>AVERRHOA BILIMBI</i>) EXTRACT ON HUMAN BREAST ADENOCARCINOMA	175
6.1	Introduction	175
6.2	Materials and Methods	179
6.2.1	Materials	179
6.2.2	Sample Preparation	179
6.2.3	Extraction	179
6.2.4	Cell Cultures	179
6.2.5	MTS Assay	180
6.2.6	Morphological Observation	180
6.2.7	Acridine Orange/Propidium Iodide Double Staining	180
6.2.8	DNA Ladder Assay	180
6.2.9	TUNEL Assay	181
6.2.10	Cell Cycle Analysis	181
6.2.11	5-Bromo-2'-deoxyuridine (BrdU) Incorporation Cell Proliferation Assay	181
6.2.12	Annexin V/Propidium Iodide (PI) Staining	182
6.2.13	Caspase-8 and -9 Assay	182
6.2.14	Caspase-3/7 Assay	182
6.2.15	Cytochrome C Assay	183
6.2.16	p53, Bax and Bcl-2 Proteins Expression	183
6.2.17	Statistical Analysis	183
6.3	Results	184
6.3.1	MTS Assay	184
6.3.2	Morphological Observation	187
6.3.3	Acridine Orange/Propidium Iodide Double Staining	190
6.3.4	DNA Ladder Assay	192
6.3.5	TUNEL Assay	194
6.3.6	Cell Cycle Analysis	196
6.3.7	5-Bromo-2'-deoxyuridine (BrdU) Incorporation Cell Proliferation Assay	204
6.3.8	Annexin V/Propidium Iodide (PI) Staining	206
6.3.9	Caspase-8 and -9 Assay	211
6.3.10	Caspase-3/7 Assay	214
6.3.11	Cytochrome C Assay	216
6.3.12	p53, Bax and Bcl-2 Proteins Expression	218
6.4	Discussion	223
6.5	Conclusion	233

7	GENERAL DISCUSSION	234
8	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	240
	8.1 Summary and General Conclusion	240
	8.2 Limitation of the Study and Recommendation for Future Research	242
	REFERENCES	243
	BIODATA OF STUDENT	267
	LIST OF PUBLICATIONS	268

