



UNIVERSITI PUTRA MALAYSIA

**NUTRITIONAL COMPOSITION AND HYPOCHOLESTEROLEMIC EFFECT
OF *CANARIUM ODONTOPHYLLUM* MIQ. FRUIT IN RABBITS**

FARIDAH HANIM BINTI SHAKIRIN

FPSK(m) 2011 38

**NUTRITIONAL COMPOSITION AND HYPOCHOLESTEROLEMIC EFFECT
OF *CANARIUM ODONTOPHYLLUM* MIQ. FRUIT IN RABBITS**



**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2011

**NUTRITIONAL COMPOSITION AND HYPOCHOLESTEROLEMIC EFFECT
OF *CANARIUM ODONTOPHYLLUM* MIQ. FRUIT IN RABBITS**



**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia in
Fulfilment of the requirements for the Master of Science**

March 2011

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

**NUTRITIONAL COMPOSITION AND HYPOCHOLESTEROLEMIC
EFFECT OF *CANARIUM ODONTOPHYLLUM* MIQ. FRUIT IN RABBITS**

By

FARIDAH HANIM BINTI SHAKIRIN

March 2011

Chairman : Azrina binti Azlan, PhD

Faculty : Medicine and Health Sciences

The main objective of this study was to determine nutrient composition, antioxidant properties of *Canarium odontophyllum* Miq. (CO) fruit and its effect on selected cardiovascular biomarkers in hypercholesterolemic and normocholesterol rabbits. Proximate composition of CO fruit pulps was determined in this study. For powdered full-fat, the fruit was rich in fat, total dietary fiber (TDF) and carbohydrate. For powdered defatted pulp, the fruit was rich in TDF and carbohydrate. CO fruit was also rich in minerals such as potassium, magnesium and calcium. Pulp and kernel oils were rich in palmitic acid and oleic acid. Total phenolic content (TPC) of samples (fruit parts and oil extracts) were determined using spectrophotometer. The TPC of the oils were in the order of; pulp oil (with skin) > pulp oil (without skin) > kernel oil. Meanwhile, the TPC of the different parts of CO was in order of skin (S) > flesh with skin (SF) > flesh (F) > kernel (K). Antioxidant capacities of the different parts of CO (S, SF, F and K) was measured using three different assay; β-carotene bleaching assay, scavenging activity on DPPH and ferric reducing/antioxidant power (FRAP) assay. The antioxidant activities of fruit extracts were in the order of: S > SF

> F > K except for DPPH assay. The lipid lowering effect of CO fruit parts was investigated in hypercholesterolemic rabbits (Study I) and normocholesterol rabbits (Study II). The parameter used in this studies are plasma lipid profile [Total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), plasma malondialdehyde (MDA), antioxidant enzymes [glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT)], total antioxidant status (TAS) and toxicity test [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT)]. The percentage of lesion of atheroma plaque was determined in this study. In Study I, supplementation of defatted pulp of CO in hypercholesterolemic rabbits showed the greatest lipid lowering effects and increased antioxidant status. The presence of high dietary fiber content and high antioxidant activity in the defatted pulp was the possible factors contributing to the retardation of atherosclerosis and reducing the risk of coronary artery disease (CHD). However, no significant effects of pulp and kernel oils of CO were found in the hypercholesterol study. Thus, the effect of oils of CO was tested in normocholesterol rabbits (Study II). In Study II, supplementation of pulp and kernel oils of CO were found beneficial in reducing the CVD risks. Consumption of pulp oil in rabbits resulted in significant increased of plasma TC and HDL-C levels and lowered plasma LDL-C level. Pulp oil resulted in increased TAS level, erythrocyte GPx and SOD activities, and reduced plasma lipid MDA levels. There were elevation in ALT, AST and GGT activities in pulp oil supplemented group as compared to control. In this study, supplementation of kernel oil of CO was found beneficial in reducing CVD risk factors as it resulted in significant reduction of plasma TC, increased HDL-C, lowered LDL-C and TG. However, the LDL-C to HDL-C ratio was significantly higher in animal fed-kernel oil compared to animal

fed normal diet. No toxic effect was found in relation to consumption of the kernel oil. As conclusion, defatted pulp and oil extracted from CO showed protective effect towards the CVD biomarkers. The protective effects could be due to high phenolic content and high antioxidant activity in the fruits.



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

**KOMPOSISI NUTRIEN DAN KESAN HIPOKOLESTEROLEMIK BUAH
CANARIUM ODONTOPHYLLUM MIQ. KE ATAS ARNAB**

Oleh

FARIDAH HANIM BINTI SHAKIRIN

Mac 2011

Pengerusi : Azrina Azlan, PhD

Fakulti : Perubatan dan Sains Kesihatan

Objektif utama kajian ini adalah untuk menentukan komposisi nutrien, aktiviti antioksida buah *Canarium odontophyllum* (CO) dan kesannya ke atas parameter kardiovaskular dalam arnab teraruh kolesterol dan arnab normokolesterol. Komposisi proksimat serbuk isi kaya lemak, isi tanpa lemak dan isi segar buah CO telah ditentukan dalam kajian ini. Berdasarkan kandungan serbuk isi kaya-lemak, buah ini kaya dengan lemak, gentian diet (TDF) dan karbohidrat. Berdasarkan serbuk isi tanpa lemak, buah ini kaya dengan TDF dan karbohidrat. Buah CO juga kaya dengan mineral seperti kalium, magnesium, dan kalsium. Kedua-dua minyak isi dan biji kaya dengan asid palmitik dan asid oleik. Jumlah kandungan fenolik dalam minyak yang diekstrak dari buah CO adalah dalam susunan: minyak isi buah (dengan kulit) > minyak isi buah (tanpa kulit) > minyak biji. Sementara itu, jumlah kandungan fenolik dalam bahagian berbeza buah CO adalah dalam susunan: kulit (S) > isi dengan kulit (SF) > isi (F) > biji (K). Kapasiti antioksida bagi bahagian-bahagian berbeza buah CO (ekstrak S, SF, F dan K), telah ditentukan melalui tiga assai berbeza; assai pelunturan β -karoten, aktiviti perencutan radikal bebas DPPH dan

kuasa penurunan/antioksida ferik (FRAP). Berdasarkan kapasiti antioksida, kulit menunjukkan aktiviti antioksida tertinggi. Aktiviti antioksida ekstrak buah tersebut adalah dalam turutan: S > SF > F > K dalam semua assai antioksida kecuali aktiviti perencatan radikal bebas (DPPH). Kesan hipokolesterolemik bahagian-bahagian buah CO telah dikaji dalam arnab teraruh kolesterol (Studi I) dan normokolesterol (Studi II). Parameter yang digunakan dalam kajian ini adalah lipid profil, lipid peroksida, enzim antioksida, status total antioksida (TAS) dan ujian toksisiti [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT)]. Peratusan pembentukan ateroma telah ditentukan dalam kajian ini. Dalam Studi 1, pemberian isi buah tanpa lemak telah menunjukkan kesan penurunan lemak paling tinggi dan peningkatan status antioksida. Kehadiran gentian diet dan aktiviti antioksida yang tinggi di dalam isi buah tanpa lemak adalah faktor yang mungkin telah menyumbang kepada perencatan atherosklerotik dan penurunan risiko penyakit koronari arteri (CHD). Walau bagaimanapun, tiada kesan signifikan terhadap pengambilan minyak isi dan biji buah CO dalam studi hiperkolesterol. Oleh itu, kesan pengambilan minyak isi dan biji telah dikaji dalam arnab normalkolesterol dalam studi II. Dalam studi ini, pengambilan minyak isi dan biji buah CO oleh arnab normokolesterol didapati berfaedah untuk mengurangkan risiko CVD (penyakit kardiovaskular). Pengambilan minyak isi menyebabkan peningkatan paras TC dan lipoprotein ketumpatan tinggi-kolesterol (HDL-C) yang signifikan. Penurunan signifikan plasma LDL-C dengan tiada perubahan signifikan nisbah LDL-C kepada HDL-C telah dikesan dalam kumpulan ini. Minyak ini telah menyebabkan peningkatan TAS secara signifikan, erittrosit GPx dan SOD dan penurunan plasma MDA. Terdapat peningkatan paras alanina transamina (ALT), aspartat transamina (AST) dan gamma-glutamyl trasamina (GGT) dalam kumpulan

minyak isi. Dalam kajian ini, minyak isi telah dikenalpasti sebagai bahan yang bermanfaat dan boleh melindungi daripada penyakit CVD kerana ia telah meningkatkan paras TAS, aktiviti enzim eritrosit GPx dan SOD dan menurunkan paras plasma MDA secara signifikan. Pengambilan minyak biji buah CO dalam arnab normokolesterol telah menyebabkan penurunan signifikan plasma TC, sedikit peningkatan HDL-C dan sedikit penurunan plasma LDL-C dan TG. Nisbah LDL-C kepada HDL-C dalam kumpulan ini telah meningkat secara signifikan berbanding kumpulan kawalan. Tiada kesan toksik ditunjukkan oleh kumpulan NK. Kesan yang baik yang ditunjukkan oleh bahagian buah CO disebabkan oleh kandungan fenolik dan aktiviti antioksida yang tinggi dalam bahagian buah tersebut.

ACKNOWLEDGEMENTS

Firstly, I would like to express my deep appreciation to my main supervisor, Dr Azrina binti Azlan for her guidance, patience, support and motivated advice throughout my study. Special thanks dedicated to my co-supervisors Associate Prof Dr Amin bin Ismail and Associate Prof Dr Zulkhairi bin Amom for good motivation, support, advice, comments and suggestion.

I also would like to convey my thankfulness to the lab staff of Department of Nutrition and Dietetics of Faculty of Medicine and Health Sciences, UPM especially, Mr. Syed Hasbullah Syed Kamaruddin and Mr. Eddy Ghadaffie bin Jamiauddin for their help, patience and guidance throughout my research study.

Deepest appreciations to my lovely husband, Muhamad Fahmi bin Hassan for his support, patience and advice. My highest gratitude to my beloved mother, Hjh Norlida binti Ismail, my sincere father, Hj Shakirin bin Omar and my siblings. Thank you to all my friends and lab mates for support, help and guidance throughout my research study.

I certify that a Thesis Examination Committee has met on 8 March 2011 to conduct the final examination of Faridah Hanim binti Shakirin on her thesis entitled "Nutritional Composition and Hypocholesterolemic Effect of *Canarium odontophyllum* Miq. Fruit in Rabbits" in accordance with the Universities and University College 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.

Members of the Thesis Examination Committee were as follows:

Zaitun binti Yassin, PhD

Associate Professor

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Asmah binti Rahmat, PhD

Professor

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Zuraini binti Hj Ahmad, PhD

Associate Professor

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Nor Fadilah Rajab, PhD

Associate Professor

Faculty of Medicine and Allied Sciences
Universiti Kebangsaan Malaysia
(External Examiner)



NORITAH OMAR, PhD

Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 June 2011

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree. The members of the Supervisor Committee are as follows:

Azrina Azlan, PhD

Lecturer

Faculty of Medicine and Health Sciences

Universiti Putra Malaysia

(Chairman)

Amin Ismail, PhD

Associate Professor

Faculty of Medicine and Health Sciences

Universiti Putra Malaysia

(Member)

Zulkhairi Amom, PhD

Associate Professor

Faculty of Medicine and Health Sciences

Universiti Putra Malaysia

(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:

DECLARATION

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

FARIDAH HANIM BINTI SHAKIRIN

Date: 8 March 2011

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDEMENT	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF ABBREVIATION	xix
 CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	8
2.1 <i>Canarium odontophyllum</i> Miq. fruit	8
2.2 Fruits and vegetables as source of antioxidants	10
2.3 Polyphenols	12
2.4 Free radical and antioxidant activities	15
2.4.1 Superoxide dismutase (SOD)	17
2.4.2 Glutathione peroxidase (GPx)	17
2.4.3 Catalase	18
2.5 LDL (Low Density Lipoprotein) oxidation	18
2.6 Atherosclerosis and its classification	19
2.7 Lipid or lipoprotein metabolism	23
2.7.1 Cholesterol	23
2.7.2 Lipoprotein	24
2.7.3 Low-density lipoprotein (LDL)	25
2.7.4 High-density lipoprotein (HDL)	25
2.7.5 Triglycerides (TG)	26
2.8 Cholesterol metabolism	27
2.9 Simvastatin	31
2.10 Hypercholesterolemia and lipid peroxidation	32
2.11 Hypercholesterolemia and toxicity	33
2.11.1 Aspartate aminotransferase	33
2.11.2 Alanine aminotransferase	33
2.11.3 Gamma-glutamyl transpeptidase (GGT)	34
2.12 Dietary fat and hypercholesterolemia	35
2.13 Dietary fiber and hypercholesterolemia	37
 3 NUTRIENT COMPOSITION AND ANTIOXIDANT CAPACITIES OF <i>C. odontophyllum</i> Miq.	39
3.1 Introduction	39
3.2 Material and methods	40
3.2.1 Sample	40
3.2.2 Chemicals	40
3.2.3 Preparation of samples	41
3.2.4 Extraction of pulp and kernel oils of CO fruit	43
3.2.5 Determination of proximate composition and mineral content	43
3.2.6 Determination of fatty acid composition of CO oils	44

3.2.7 Determination of total phenolic content in pulp and kernel oils	45
3.2.8 Determination of total phenolic content and antioxidant activity of CO fruit parts	46
3.2.9 Statistical analysis	50
3.3 Results and discussion	50
3.3.1 Proximate composition, dietary fiber and mineral content	50
3.3.2 Fatty acid composition	55
3.3.3 Total phenolic content in oils	58
3.3.4 Total phenolic content of different parts of CO fruit	60
3.3.5 The antioxidant capacity of different parts of CO fruit	61
3.3.6 Correlation between total phenolic content and antioxidant capacities	66
3.4 Conclusions	66
4 CHOLESTEROL LOWERING EFFECT OF <i>C. odontophyllum</i> Miq. IN HYPERCHOLESTOLEMIC RABBITS	68
4.1 Introduction	68
4.2 Materials and methods	69
4.2.1 Fruit and experimental animals	69
4.2.2 Animals diets	69
4.2.3 Chemicals and reagent	69
4.2.4 Preparation of fruit	70
4.2.5 Extraction of oil from pulp and kernel of <i>C. odontophyllum</i> fruit	70
4.2.6 Experimental design	70
4.2.7 Preparation of animal diets	72
4.2.8 Preparation of 0.5% cholesterol diet	74
4.2.9 Food intake and body weight	77
4.2.10 Blood collection	77
4.2.11 Lipid profile analysis	78
4.2.12 Lipid peroxidation	80
4.2.13 Antioxidant status	81
4.2.14 Toxicity profile	84
4.2.15 Atheroma plaque scoring	86
4.3 Statistical analysis	87
4.4 Results	88
4.4.1 Food intake and body weight	88
4.4.2 Lipid profile	91
4.4.3 Malondialdehyde (MDA) level	101
4.4.4 Antioxidant status	103
4.4.5 Toxicity study	111
4.4.6 Assessment of Atherosclerotic plaques	117
4.5 Discussion	122
4.5.1 Food intake and body weight	122
4.5.2 Lipid profile	123
4.5.3 Malondialdehyde (MDA)	128
4.5.4 Antioxidative mechanism	129
4.5.5 Toxicity	133

4.5.6 Atheroma plaque	135
4.5.7 Conclusions	138
5 CHOLESTEROL LOWERING EFFECT OF PULP OIL AND KERNEL OILS EXTRACTED FROM <i>C. odontophyllum</i> Miq. FRUIT IN NORMOCHOLESTEROL RABBITS	140
5.1 Introduction	140
5.2 Materials and methods	141
5.2.1 Fruit and experimental animals	141
5.2.2 Animal diets	141
5.2.3 Chemicals and reagents	141
5.2.4 Fruit preparation	141
5.2.5 Oil extraction from the pulp and kernel of CO	142
5.2.6 Experimental animals	142
5.2.7 Preparation of experimental diets	144
5.2.8 Food intake and body weight	146
5.2.9 Blood collection	146
5.3 Results	147
5.3.1 Food intake and body weight	147
5.3.2 Effect of CO oils on plasma lipid profiles	148
5.3.3 Effect of CO oils on plasma lipid peroxidation and antioxidative status	152
5.3.4 Toxicity effect of CO oils	155
5.4 Discussion	157
5.4.1 Food intake and body weight	157
5.4.2 Effect of CO oils on plasma lipid profile	158
5.4.3 Effect of CO oils on plasma lipid peroxidation marker	160
5.4.4 Effect of CO oils on antioxidant status	162
5.4.5 Effect of CO oils on liver function tests	164
5.5 Conclusions	165
6 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	166
6.1 Summary	166
6.2 General conclusions	166
6.3 Limitation of study	168
6.4 Recommendation for future research	168
REFERENCES	170
APPENDICES	199
BIODATA OF STUDENT	207
LIST OF PUBLICATIONS	208