



EXTRACTION OF FUCOXANTHIN FROM
SELECTED MALAYSIAN BROWN
SEAWEEDS AND ITS EFFECTS ON 3T3-L1
ADIPOCYTE CELLS

BY

HASNA AHMAD

A thesis submitted in fulfilment of the requirement for
the degree of Masters of Science (Biotechnology)

Kulliyyah of Science
International Islamic University Malaysia

FEBRUARY 2017

ABSTRACT

Obesity (BMI >30 kg/m²) is becoming a major public problem as it affects over 400 million of the population. Despite advances in the development of more effective weight loss drugs, safety and dependence remains an issue. This study aims to establish the antiobesity potential of Malaysian brown seaweeds *Sargassum oligocystum*, *Padina australis* and *Dictyota dichotoma*. Fucoxanthin has been described as the main bioactive compound in brown seaweed exerting antiobesity effect. Three extraction parameters were optimized in order to retain maximum fucoxanthin content in the extract: solvent, particle size and method of extraction. Optimal solvent and particle size for extraction across all seaweeds was acetone and particles sieved through 500µm mechanical sieve. *S. oligocystum* and *P. australis* were best extracted using Soxhlet procedures, while *D. dichotoma* was best extracted using maceration. The fucoxanthin content was analyzed using HPLC equipment. The highest amount of fucoxanthin present in the seaweeds was found in *Sargassum oligocystum* (754.8 mg/g dry weight), followed by *D. dichotoma* (142.9 mg/g) and finally *P. australis* extracts (91.58 mg/g). The extract showing best antiadipogenic effect on the preadipocytes was SAE (lipid accumulation equivalent to 0.170 ± 0.034 A), followed by DAE (0.196 ± 0.082 A) and finally PAE (0.203 ± 0.047 A). SAE had the best proadipolytic effect on mature lipid cells (31.86 ± 17.56 mg/ml glycerol release), followed by PAE (30.54 ± 15.41 mg/ml) and finally DAE (28.12 ± 11.36 mg/ml). Statistical analysis using one- way ANOVA indicated that the differences were not significant ($p > 0.05$). Overall, antiobesity activity levels did not coincide with fucoxanthin levels present in the extract, indicating the presence of various compounds in the extract that exert diverse effects on 3T3-L1 adipocytes. The study describes fucoxanthin content of the Malaysian seaweeds and its optimal extraction parameters. Furthermore, it indicates the adipogenic and adipolytic potential of the extracts as antiobesity agents and the potential for seaweed extracts to be further developed as a safe and effective natural alternative for the management of obesity.

خلاصة البحث

أصبحت السمنة المفرطة (مؤشر كتلة الجسم أو BMI أعلى من 30 كجم/م²) من المشاكل الاجتماعية الكبرى والتي تؤثر على حوالي 400 مليون نسمة من التعداد السكاني. على الرغم من التقدم العلمي في تطوير عقاقير تخفيض الوزن فلا زالت أمور السلامة والإدمان من المشاكل المتعلقة بها. هدفت هذه الدراسة إلى إثبات الخواص المضادة للسمنة للأعشاب البحرية البنية الماليزية، وهي من نوع سارجسم أوليقوسستم، وبادينا أوسترليس، وديكيتيتوتا دايكوتوما. تم التعرف على أن مركب فوكوزانتين هو المركب النشط حيويًا الأساسي في الأعشاب البحرية البنية المتميزة بالخواص المضادة للسمنة. تم تحسين مؤشرات عملية الاستخلاص للحفاظ على أعلى نسبة من الفوكوزانتين في المستخلصات، وهي نوع المذيب، حجم الذرة، وطريقة الاستخلاص. نوع المذيب وحجم الذرة المناسبين لاستخلاص الأعشاب البحرية كان الأسيتون، والذرات المصفاة خلال مصفاة من قياس 500 ميكرومتر. أفضل طريقة لاستخلاص سارجسم أوليقوسستم وبادينا أوسترليس كان بواسطة السوكسلت، والديكيتيتوتا دايكوتوما بواسطة النقع. تم حساب كمية الفوكوزانتين بواسطة جهاز التحليل الكروماتوجرافي العالي الكفاءة والذي وجد بأعلى كمية في سارجسم أوليقوسستم (الوزن الجاف 754.8 مغ/غ)، ومن ثم الديكيتيتوتا دايكوتوما (142.9 مغ/غ)، وأخيرا وبادينا أوسترليس (91.58 مغ/غ). أفضل نشاط ضد السمنة على الخلايا الماقبل الدهنية كان من قبل السارجسم أوليقوسستم (تراكم الدهون المعادل ل 0.034 ± 0.170 A)، ومن ثم الديكيتيتوتا دايكوتوما (0.082 ± 0.196 A) وأخيرا البادينا أوسترليس (0.047 ± 0.203 A). أظهر السارجسم أوليقوسستم أفضل تحلل للخلايا الماقبل الدهنية وعلى الخلايا الدهنية المكتملة (17.56 ± 31.86 مغ/مل كمية إطلاق الجلوسول)، ومن ثم البادينا أوسترليس (15.41 ± 30.54 مغ/مل)، وأخيرا الديكيتيتوتا دايكوتوما (11.36 ± 28.12 مغ/مل). أظهر تحليل التباين الأحادي الأنوفا أن الفروق ليست مهمة نسبيًا ($p > 0.05$). بشكل عام، مستويات النشاط المضاد للسمنة لم تتناسب مع مستويات الفوكوزانتين المتواجدة في المستخلصات، مما يشير إلى أن هنالك العديد من المركبات الأخرى المؤثرة بشكل مختلف على خلايا 3T3-L1 الدهنية. وصفت الدراسة محتوى الفوكوزانتين في الأعشاب البحرية الماليزية وطرق استخلاصها المثلى. بالإضافة إلى ذلك، أشارت هذه الدراسة إلى الخواص المكونة للشحوم والخواص المضادة للشحوم للمستخلصات المحتملة كمركبات مضادة للسمنة واحتمال تطوير هذه الأعشاب في كبدايل طبيعية وآمنة لتنظيم السمنة.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology).

.....
Irwandi Jaswir
Main Supervisor

.....
Deny Susanti
Co- Supervisor

.....
Muhammad Taher
Co- Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology)

.....
Muhammad Nor Omar
Internal Examiner

.....
Tan Chin Ping
External Examiner

This thesis was submitted to the Department of Biotechnology and is accepted as a fulfilment of the requirements for the degree of Master of Science (Biotechnology)

.....
Suhaila Mohd Omar
Head, Department of
Biotechnology

This thesis was submitted to the Kulliyah of Science and is accepted as a fulfilment of the requirements for the degree of Master of Science (Biotechnology)

.....
Kamaruzzaman Yunus
Dean, Kulliyah of Science

DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Hasna Ahmad

Signature.....

Date

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF
FAIR USE OF UNPUBLISHED RESEARCH**

**EXTRACTION OF FUcoxANTHIN FROM SELECTED
MALAYSIAN BROWN SEAWEEDS AND ITS EFFECTS ON
3T3-L1 ADIPOCYTE CELLS**

I declare that the copyright holders of this thesis are jointly owned by the student and
IIUM.

Copyright © 2017 Hasna Ahmad and International Islamic University Malaysia. All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval system,
or transmitted, in any form or by any means, electronic, mechanical, photocopying,
recording or otherwise without prior written permission of the copyright holder
except as provided below

1. Any material contained in or derived from this unpublished research may
be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies (print
or electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieved system
and supply copies of this unpublished research if requested by other
universities and research libraries.

By signing this form, I acknowledge that I have read and understand the IIUM
Intellectual Property Right and Commercialization policy.

Affirmed by Hasna Ahmad

.....
Signature

.....
Date

ACKNOWLEDGMENTS

First of all, I would like to express my gratitude to Allah SWT for it is first and foremost with His permission and will that I am able to start and end this journey.

Alhamdulillah, I am grateful to my supervisor Prof. Dr. Irwandi Jaswir, who has encouraged me with his strength of character, knowledge and integrity throughout the study. I would like to also thank my co- supervisors Dr. Deny Susanti and Dr. Muhammad Taher Bakhtiar who have advised me and guided me from the beginning to the end of the study. I would also like to thank Prof. Dr. Ahmed Jalal Khan Chowdhury, Deputy Dean for Postgraduate and Research Kulliyah of Science for it is with his recommendation, generosity and kindness that I was able to embark on this journey.

Here I would also like to extend my thanks to my parents, who supported me indefinitely throughout this study period, and bearing with my absence. Special mention goes to my siblings who have been patient, strong and understanding during this time away from them.

I shall not forget to acknowledge also the people who have extended me much professional help throughout this journey- administrative staff of the Kulliyah of Science with special mention to Sr. Norsa'adah binti Md Yunos; laboratory staff of the Kulliyah of Science including Br. Ahmad Muzzammil bin Zuberdi, Br. Mohd Lazuardi Ilham, Br. Mohamad Romizan bin Osman; laboratory staff of the Kulliyah of Pharmacy including Sr. Hazan Haryanti binti Abdul Halim and Sr. Zalilah binti Md Tahir.

Most importantly, all this would not have been possible without the constant motivation, camaraderie and help from my colleagues and friends whose suggestions, friendship and support kept me going through the difficult times.

May Allah bless and reward all of them in return.

TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic.....	iii
Approval page	iv
Declaration	v
Copyright Page.....	vi
Acknowledgement	vii
List of Tables.....	xi
List of Figures	xiii
List of Equations.....	xv
List of Symbols	xvi
List of Abbreviations	xvii
CHAPTER ONE: INTRODUCTION	1
1.1 Background of the Research.....	1
1.2 Scope and Objectives	3
1.3 Hypothesis	3
1.4 Brief Explanation on the Flow of Experiments	3
CHAPTER TWO: LITERATURE REVIEW	7
2.1 Obesity	7
2.1.1 Introduction	7
2.1.2 Prevalence of Obesity in Malaysia	8
2.1.3 Understanding Obesity.....	10
2.1.3.1 Obesity and the Adipose Tissue	10
2.1.3.2 Adipocyte Cell Biology	11
2.1.3.3 Adipolysis	12
2.1.3.4 Apoptosis	14
2.1.3.5 3T3-L1 Cells	14
2.1.4 Treatment of Obesity	16
2.1.4.1 Pharmacotherapy and Surgery	16
2.1.4.2 Natural Medicine as an Alternative for the Treatment of Obesity.....	18
2.2 Malaysian Seaweeds	19
2.2.1 <i>Sargassum oligocystum</i>	21
2.2.2 <i>Padina australis</i>	22
2.2.3 <i>Dictyota dichotoma</i>	23
2.3 Fucoxanthin	25
2.3.1 Metabolism and Accumulation.....	28
2.3.2 Cytotoxicity and Mutagenicity of Fucoxanthin.....	28
2.3.3 Antiobesity Activity of Fucoxanthin	28
2.3.3.1 In vivo Studies.....	28
2.3.3.2 In vitro Studies	29
2.3.3.3 Antiobesity Activity of Fucoxanthin Metabolites and Combinations	30
2.4 Extraction of Fucoxanthin	30

CHAPTER THREE: METHODOLOGY	33
3.1 Materials	33
3.1.1 General Instrumentation	33
3.1.2 Chemicals	33
3.2 Procurement of Plant	34
3.3 Procurement of Cell Line	34
3.4 Experimental Procedures.....	34
3.4.1 Sample Preparation	34
3.4.2 Plant Extraction	35
3.4.2.1 Experimental Design	35
3.4.2.2 Solvent Type	36
3.4.2.3 Extraction Method	37
3.4.2.4 Particle Size.....	37
3.4.3 HPLC Analysis of Fucoxanthin Content in Extract	37
3.4.3.1 Standard Curve Preparation	37
3.4.3.2 Sample Analysis	37
3.4.4 Cell Culture Protocols.....	39
3.4.4.1 Cell Maintenance	39
3.4.4.2 Cell Counting and Viability	39
3.4.4.3 Cell Growth.....	40
3.4.5 Cell Viability Assay /MTT Assay	40
3.4.6 Adipogenesis and Adipolysis	41
3.4.6.1 Sample Preparation.....	41
3.4.6.2 Adipogenesis	42
3.4.6.3 Adipolysis Assay Protocol.....	43
3.5 Statistical Analysis.....	45
 CHAPTER FOUR: RESULTS AND DISCUSSION	 46
4.1 Preamble.....	46
4.2 Optimization Of Solvent Extraction For Fucoxanthin Content In Brown Seaweed Extract	47
4.2.1 <i>Sargassum oligocystum</i>	49
4.2.2 <i>Padina australis</i>	52
4.2.3 <i>Dictyota dichotoma</i>	54
4.2.4 Yields of Extract.....	56
4.2.5 Acetone as Optimal Solvent.....	57
4.2.6 Intermediate Particle Size as Optimal Size	59
4.2.7 Extraction Method Affects Fucoxanthin Content in Whole Extract	59
4.2.8 Interaction Effects of Variables.....	60
4.3 Antiobesity Potential of Extracts	61
4.3.1 Effect on Adipogenesis	61
4.3.2 Effect on Adipolysis	64
4.3.3 SPSS Analysis	66
4.4 Fucoxanthin Levels and Antiobesity Activity.....	69
 CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS	 71
 REFERENCES	 73

APPENDIX 1	91
APPENDIX 2	96
APPENDIX 3	97
APPENDIX 4	103
APPENDIX 5	106
APPENDIX 6	112

LIST OF TABLES

Table 2.1	Nutritional Status Indicator, Classification and Cutoff Points (Suzana et al., 2010)	23
Table 2.2	Bioactivity reported in fucoxanthin	40
Table 2.3	Brown seaweeds reported in fucoxanthin extraction	45
Table 3.1	Experimental parameters in the design	49
Table 3.2	Boiling point of solvents used	50
Table 3.3	RP-HPLC Parameters for Analysis of Fucoxanthin Content	51
Table 3.4	Seeding densities used for different cell culture flasks/ 96 well plate	54
Table 3.5	Media used for cell adipogenesis	56
Table 3.6	Medium used for cell adipolysis	59
Table 4.1	HPLC area and amounts generated using Chromera software	63
Table 4.2	HPLC area and amounts generated using Chromera® software.	65
Table 4.3	Fucoxanthin content (mg/ml) of <i>Sargassum oligocystum</i> extracts under different extraction conditions. Values are mean of triplicates.	67
Table 4.4	Fucoxanthin content (mg/ml) of <i>Padina australis</i> extracts under different extraction conditions. Values are mean of triplicates	73
Table 4.5	Fucoxanthin content (mg/ml) of <i>Dictyota dichotoma</i> extracts under different extraction conditions. Values are mean of triplicates.	74
Table 5.1	Maximum fucoxanthin content in three seaweed extracts extracted under different conditions	79

Table 5.2	Percentage increase in fucoxanthin content, under optimized extraction conditions	80
Table 5.3	Fucoxanthin concentration in extracts (mg/ml)	82
Table 5.4	Fucoxanthin content of some species of brown seaweeds from Malaysia	87

LIST OF FIGURES

Figure 1.1	General flow chart of experiment	20
Figure 2.1	Overweight and obesity rates in Malaysia for the years 1996 and 2011. Both classifications increased over a time period of 15 years.	24
Figure 2.2	The adipocyte differentiation or adipogenesis process and important factors (Nagy, Bálint, Bálint L., Meskó, Lányi, Scholtz, Széles, Varga; 2011)	26
Figure 2.3	Lipolysis in the removal of lipids from the body (Frayn, Bernard, Spalding, Arner; 2012)	27
Figure 2.4	Mature adipocytes	30
Figure 2.5	<i>Sargassum oligocystum</i>	37
Figure 2.6	<i>Padina australis</i> Hauck	38
Figure 2.7	<i>Dictyota dichotoma</i>	39
Figure 2.8	Fucoxanthin structure	40
Figure 3.1	Order of solvents used, in increasing polarity	50
Figure 3.2	Flowchart of plant preparation till analysis of extract using HPLC from brown seaweeds	52
Figure 3.3	Flowchart of adipogenesis and adipolysis assays using fucoxanthin- rich extracts SAE, PAE and DAE	59
Figure 4.1	Standard curve for fucoxanthin standard	61
Figure 4.2	HPLC chromatogram for <i>D. dichotoma</i> extracted using acetone at room temperature, particle size 500 µm. Fucoxanthin was detected at Maceration 3.228 min.	62
Figure 4.3	HPLC chromatogram for <i>S. oligocystum</i> extracted using methanol at room temperature, particle size 500 µm. Fucoxanthin was detected at Maceration 3.220 min.	62
Figure 4.4	Maximum and minimum fucoxanthin content in <i>Sargassum</i>	65

oligocystum extracts using different extraction parameters

Figure 4.5	Comparison between maximum and minimum fucoxanthin content in <i>Padina australis</i> extracts using different extraction parameters	67
Figure 4.6	Comparison between maximum and minimum fucoxanthin content in <i>Dictyota dichotoma</i> extracts using different extraction parameters	69
Figure 4.7	Fibroblast- like preadipocyte cells at 70% confluence	70
Figure 4.8	Differentiated preadipocytes (x10 magnification)	71
Figure 4.9	Differentiated preadipocytes stained with Oil Red O stain (x10 magnification)	71
Figure 4.10	Differentiated preadipocytes stained with Oil Red O stain (x20 magnification)	72
Figure 4.11	Undifferentiated preadipocyte cells (x10 magnification)	72
Figure 4.12	Glycerol standard curve. Graph shows absorbance readings as a function of glycerol concentration.	74
Figure 5.1	Oil red O staining (A490) quantities after treatment with extracts and controls. Higher staining indicates higher degree of 3T3-L1 cell differentiation	90
Figure 5.2	Lipid metabolism measured as glycerol concentration ($\mu\text{g/ml}$) present in the media after treatment	93
Figure 5.3	Fucoxanthin content in the seaweed extracts, in increasing order	95
Figure 5.4	Anti-adipogenic effect of the seaweeds, in increasing order	95
Figure 5.5	Pro-adipolytic effect of the seaweeds, in increasing order	95

LIST OF EQUATIONS

Equation 4.1	Yield of extracts	49
Equation 4.2	Total yield of fucoxanthin (mg/g dry weight)	50
Equation 4.3	Glycerol concentration	65

LIST OF SYMBOLS

μm	Micrometer
cm	Centimeter
cm^2	Centimeter squared
g	Grams
h	Hours
kg	Kilograms
Kg/m^2	Kilograms per square meter
m	Meters
m^2	Square meters
mg/g	Milligram per gram
min	Minutes
ml	Milliliters
ml/min	Milliliters per minute
mm	Millimeter
nm	Nanometer
Å	Angstrom
Ac	Acetone
CO_2	Carbon dioxide
D0	Day zero
D2	Day two
D4	Day four
D7	Day seven
pH	Potential hydrogen

LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
ATCC	American Type Cell Culture
BMI	Body Mass Index
CEBP	CCAT enhancer binding protein
CAMP	Cyclic adenosine monophosphate
CREB	Cyclic response element binding
DAE	<i>D. dichotoma</i> acetone extract
DMX	Dexamethasone
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
et al.	Et alia
EtOH	Ethanol
FAO	Food and Agriculture Organization
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFA	Free fatty acids
Fx	Fucoxanthin
HPLC	High performance liquid chromatography
IBMX	Isobutylmethylxanthine
IGF1	Insulin growth factor 1
LLE	Liquid- liquid extraction

M	Mean
MDI	Methylisobutylxanthine, dexamethasone, insulin
MeOH	Methanol
Mets	Metabolic syndrome
mRNA	Messenger ribonucleic acid
ORO	Oil red O
PAE	<i>P. australis</i> acetone extract
PBS	Phosphate buffered saline
PPAR- γ	Peroxisome proliferator activated receptor gamma
PUFA	Polyunsaturated fatty acids
RAPD	Random amplified polymorphic DNA
RP	Reverse phase
Rt	Retention time
SD	Standard deviation
SLE	Solid liquid extraction
SPSS	Statistical Package for Social Sciences
TNF- α	Tumor Necrosis Factor alpha
UCP	Uncoupling protein
UK	United Kingdom
UV	Ultraviolet
WAT	White adipose tissue
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE RESEARCH

Obesity is fast becoming the next preventable epidemic in the world, with over 11.6 billion of the population being overweight (BMI > 25 kg/m²), and 400 million more obese (BMI > 30 kg/m²) (WHO, 2013). Prevalence has been rising, including in developing and underdeveloped countries, with numbers forecasted to increase by 33 % in 2030 (Finkelstein, Khavjou, Thompson, Trogon, Pan, Sherry, Dietz; 2012). This trend is worrying as obesity has been reported as a risk factor for several diseases including high blood pressure, diabetes, cardiovascular disease, several cancers, disabilities and death (WHO, 2013; Reilly, Methven, Mcdowell, Hacking, Alexander, Stewart, Kelnar; 2003). The healthcare costs associated with the treatment of these diseases has also seen a worrying rise. In 2006 alone, healthcare expenditure was estimated at 100 billion dollars per year and is expected to rise to approximately 500 billion dollars per year in 2030 (WHO, 2013; Finkelstein et al., 2012). It has been reported that the in addition to the increased risk for metabolic disease, quality of life of obese/overweight patients is lower than the average individual, owing to the discrimination, stigma and prejudice they face (Puhl & Heuer, 2009; Jia & Lubetkin, 2005). All these factors combined have resulted in obesity becoming a serious global healthcare issue.

Several methods are prescribed in the management of obesity, including mild lifestyle changes, to the use of antiobesity drugs, to invasive surgery in chronic cases. It has been reported that diet and exercise do not result in sustained weight loss

(Douketis, Macie, Thabane, Williamson; 2005); therefore pharmacotherapy remains an interesting option for the management of obesity. However, despite advances in the development of more effective weight loss drugs that result in sustained, meaningful weight loss, safety and dependence remains an issue. Amidst approval for new weight loss drugs such as Contrave® in 2014, regulatory bodies such as the Food and Drug Administration (FDA) have warned that such drugs have the potential for abuse, and increase the risk of serious suicidal thoughts, seizures and hypertension in some patients (FDA, 2014). Natural products have long been an alternative to drugs in the treatment of diseases (Newman & Cragg, 2012). This makes the search for effective and safe weight loss prescriptions sourced from natural products an attractive pursuit for the management and treatment of obesity, as previous studies have reported (Vasudeva, Yadav, Sharma, 2012; Han, Kimura, Okuda, 2005; Mohamed, Ibrahim, Elkhayat, El Dine, 2014; Yun, 2010).

Malaysia is blessed with high biodiversity on land and in the sea which may be exploited to discover new natural products. This study focuses on three brown seaweeds harvested from Malaysian waters *Sargassum oligocystum*, *Padina australis* and *Dictyota dichotoma*. The main bioactive compound reportedly contributing to antiobesity activity in brown seaweeds is the carotenoid fucoxanthin (Maeda et al., 2005, 2006). This study aimed to establish the antiobesity potential of selected Malaysian brown seaweeds *Sargassum oligocystum*, *Padina australis* and *Dictyota dichotoma* via their action on adipocyte differentiation (adipogenesis) and metabolism (adipolysis) and to optimize extraction parameters to retain maximum fucoxanthin content in the extract.

1.2 SCOPE AND OBJECTIVES

The study focused on the anti-obesity potential of selected Malaysian brown seaweeds *Sargassum oligocystum*, *Padina australis* and *Dictyota dichotoma* harvested from the west coast of Port Dickson via adipogenesis and adipolysis. Optimization steps for high extraction yields of the target compound, fucoxanthin were also studied. The specific objectives were:

- To determine optimal solvent, particle size and method of extraction of brown seaweeds to achieve high yields of fucoxanthin
- To determine the effect of fucoxanthin-rich extracts of seaweed on adipogenesis in 3T3-L1 cells
- To determine the effect of fucoxanthin-rich extracts of seaweeds on adipolysis in 3T3-L1 cells
- To link between fucoxanthin content and antiobesity activity present in the seaweed extracts

1.3 HYPOTHESIS

Optimal parameters for high retention of fucoxanthin in the seaweed would be different according to the nature of the seaweed. Malaysian brown seaweed extracts containing fucoxanthin would exert anti-adipogenic and pro-adipolytic activities towards 3T3-L1 cells.

1.4 BRIEF EXPLANATION ON THE FLOW OF EXPERIMENTS

Briefly, the study consisted of two major parts: firstly, optimizing the extraction process of the brown seaweeds to ensure high retention of fucoxanthin and secondly,

applying the extracts to 3T3-L1 fat cells to establish the adipogenic and adipolytic effects on the locally sourced brown seaweeds.

Fucoxanthin is the main compound reported to be responsible for anti-adipogenic activity in brown seaweeds, as reported by previous studies (Maeda, Hosokawa, Sashima, Takahashi, Kawada, Miyashita; 2006; Maeda, Hosokawa, Sashima, Funayama, Miyashita; 2005). Therefore, an important consideration during extraction is to ensure that fucoxanthin is retained in the extract during processing. In the first part of this study, important parameters were controlled to optimize extraction: solvent, particle size used and extraction method. Up to three solvents with different degrees of polarity were used: acetone, methanol and ethanol. Two methods were used for extraction: Soxhlet extraction and room temperature maceration ($26\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). Different particle sizes were achieved after grinding by sieving the dried plant powder and passed through different mechanical sieve sizes: 125 μm , 500 μm and 1000 μm (1 mm). Fucoxanthin in the extracts was quantified using HPLC. The results were tabulated and analysed using SPSS. Extracts containing the highest fucoxanthin content from each type of brown seaweed was selected for the second part of the study.

The second part of the study involved investigating the effect of the extracts from different Malaysian brown seaweeds on 3T3-L1 cells. 3T3-L1 cells are well-established models for understanding lipid metabolism. Adipogenesis assay was carried out using an adipogenesis assay kit. Extracts were applied during the differentiation stage to study the effect of extracts on preadipocyte differentiation. The cells were stained with Oil Red O and cell differentiation was quantified using a microplate reader. Meanwhile, adipolysis assay was carried out on differentiated cells to study the effect of the extracts on release of triglycerides from the cells. The

preadipocytes were allowed to mature before application of extracts. Glycerol release in the cell media was quantified using a microplate reader. Suitable concentrations of extracts for the study were determined using MTT assay. Cells were treated at different concentrations of the extract to determine the cutoff point to ensure cell viability. A general flowchart of the entire research is presented in Figure 1.1.

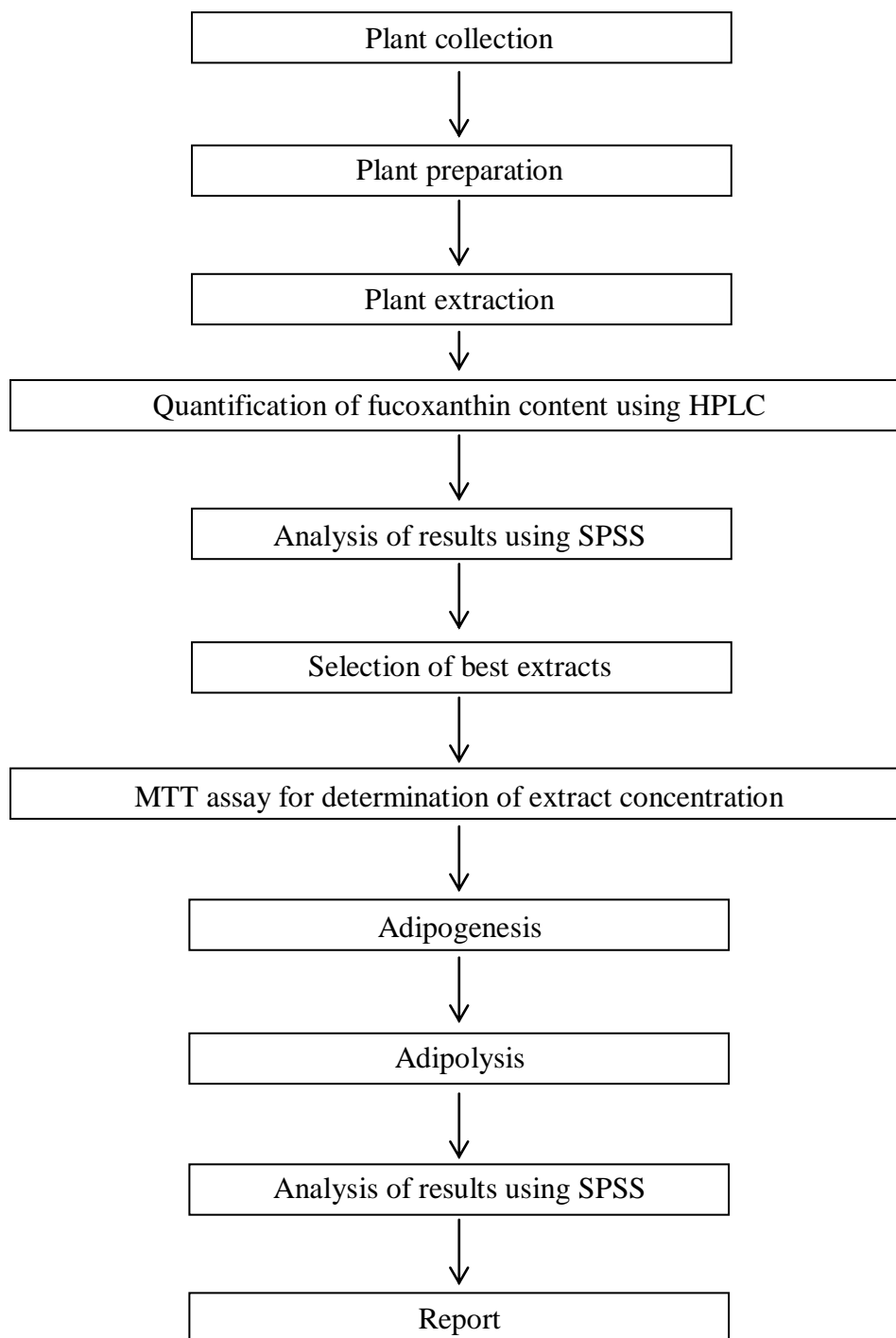


Figure 1.1 General flow chart of experiment