



**EFFECT OF CUCURBITACEAE EXTRACTS ON GLUCOSE
AND LIPID METABOLISM IN 3T3-L1 ADIPOCYTES**

BY

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ABSTRACT

The use of herbal remedies in the prevention of disease conditions has been the focus of many studies due to their multiple health benefits. One such condition is a state of hyperglycemia which may be altered with the use of natural products originating from herbs, fruits or vegetables. Three of the proclaimed vegetables were used in this study, a commonly consumed gourds belonging to the *Cucurbitaceae* family, namely: *Momordica charantia* (also known as Bitter gourd, Karela or Peria Katak), *Trichosanthes cucumerina* (known as Snake gourd or Labu Ular) and *Lagenaria siceraria* (known as Bottle Gourd, Opo squash or Calabash). Water and ethanol extracts from whole vegetable, peels and seeds were assessed for antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl assay (DPPH assay), total phenolic assay (TPC assay) and total flavonoid assay (TFC). The total phenolic contents were highest in the ethanol extract of *Lagenaria Siceraria* peels (LSPe), the water extract of *Momordica charantia* seeds (MCSw) and the ethanol extract of *Trichosanthes cucumerina* peels (TCPe) being 35.58 ± 1.2 , 33.03 ± 1.4 and 20.70 ± 1.26 mg GAE/g, respectively. Total flavonoid contents were highest in the seeds of all three vegetables for the ethanol extract of *Lagenaria Siceraria* seeds (LSSe), the water extract of *Trichosanthes cucumerina* seeds (TCSw) and the ethanol extract of *Momordica charantia* seeds (MCSe) being 0.73 ± 0.01 , 0.67 ± 0.02 and 0.65 ± 0.02 mg QE/g, respectively in accordance with the high total phenol content. The extracts exhibited high free radical scavenging activity with the highest inhibition for DPPH being 79.80, 73.92 and 71.97% for LSPe, TCPe and MCSw, respectively. Cell viability assay for water and ethanol extracts revealed that 3T3-L1 adipocytes maximum toleration concentration was 0.063 mg/ml. The extracts were further tested on adipocytes' differentiation and showed a stimulation of lipid droplets formation during adipogenesis. The extracts significantly ($p < 0.001$) increased glycerol release levels during adipolysis compared to the control and were higher in all three sources for the ethanol extracts of *Trichosanthes cucumerina* whole vegetable (TCWe), *Lagenaria siceraria* whole vegetable (LSWe) and *Momordica charantia* peels (MCPe), respectively. The extracts also significantly ($p < 0.001$) promoted the uptake of glucose in the cells for the ethanol extract of *Lagenaria siceraria* whole vegetable (LSWe), ethanol extract of *Trichosanthes cucumerina* whole vegetable (TCWe) and water extract of *Momordica charantia* seeds (MCSw) for up to 81.31, 71.24 and 56.18%, respectively. The effect of the extracts was further tested on adiponectin and leptin concentrations in adipocytes using ELISA kit assay where it indicated a significant ($p < 0.01$) increase in adiponectin concentrations for the water extract of *Lagenaria siceraria* peels (LSPw), the water extract of *Momordica charantia* peels (MCPw) and the water extract of *Trichosanthes cucumerina* seeds (TCSw) with 5405 ± 1557 , 4966 ± 1097 and 3593 ± 225 ng/ml, respectively and a decrease in leptin concentrations for the extracts LSPw and MCSe with 14633 ± 612 and 17578 ± 1536 pg/ml, respectively. The present study data showed that there is a beneficial effect of the extracts on adipogenesis, adipolysis and glucose uptake in 3T3-L1 adipocytes. Furthermore, the observed adipocytes concentrations of adiponectin and leptin could be of clinical importance in energy regulation which is a key factor in diabetes, obesity and metabolic syndrome.

خلاصة البحث

استخدام العلاجات العشبية في الوقاية من الحالات المرضية يعتبر محورا للعديد من الدراسات بسبب فوائدهم الصحية المتعددة. أحد هذه الحالات هو حالة ارتفاع السكر في الدم التي يمكن أن تتغير مع استخدام المنتجات الطبيعية التي تستخلص من الأعشاب والفواكه أو الخضروات. ثلاثة من الخضروات تم استخدامها في هذه الدراسة، القرع الذي يشيع تناوله ينتمي للعائلة القرعية، وهي: قرع مر (*Momordica charantia*) (المعروف أيضا باسم القرع المر، كاريل أو بيريا كاتاك، *Trichosanthes cucumerina*) (المعروفة باسم الأفعى القرع أو لايو أولار) و (*Lagenaria siceraria*) (المعروفة باسم زجاجة القرع، لايو سكواش أو كالاباش). تم الاستخلاص باستخدام الماء والايثانول (الاستخلاص من الخضروات كلها، القشور والبذور) للأنبشطة المضادة للأكسدة باستخدام فحص 2، 2-ثنائي- picrylhydrazyl فحص (DPPH)، ومجموع الفينول (فحص TPC) ومجموع فحص الفلافونويد (TFC). بلغ إجمالي محتويات الفينول الأعلى في مستخلص الإيثانول من القشور (*Lagenaria siceraria* (LSPe)، ومستخلص المياه من بذور القرع المر (MCSw) ومستخلص الإيثانول من القشور *Trichosanthes cucumerina* (TCPe) كانت 1.2 ± 35.58 ، 1.4 ± 33.03 و 1.26 ± 20.70 مليجرام /GAE جرام على التوالي. وبلغ إجمالي محتويات الفلافونويد أعلى في بذور جميع الخضروات الثلاثة لمستخلص الإيثانول من البذور (*Lagenaria siceraria* (LSSe)، ومستخلص المياه من البذور *Trichosanthes cucumerina* (TCSw) ومستخلص الإيثانول من بذور القرع المر (MCSE) كانت 0.01 ± 0.73 ، 0.02 ± 0.67 و 0.02 ± 0.65 مليجرام / QE جرام على التوالي. أظهرت المستخلصات ارتفاعا عاليا في نشاط الكسح للجذور الحرة وفقا لأعلى تثبيط DPPH حيث كانت 79.80 ، 73.92 و 71.97% لل LSPe، TCPe و MCSw على التوالي. كشف فحص نمو الخلايا للمستخلصات المائية والايثانول أن تركيز الخلايا الدهنية أقصى تركيز للتحميل 0.063 مليجرام / مليلتر. تم اختبار مستخلصات أخرى على تمايز الخلايا الدهنية وأظهرت تحفيز تشكيل قطرات الدهون أثناء تكون الشحم. ارتفعت مستويات تركيز الجلسرول ارتفاعا ملحوظا ($P < 0.001$) خلال تحليل الدهون مقارنة بالمجموعة الضابطة وكانت أعلى في جميع المصادر الثلاثة للمستخلص الإيثانول من *Trichosanthes cucumerina* الخضار كلها (TCWe)، *Lagenaria siceraria* الخضار كلها (LSWe) وقشور القرع المر (MCPe) على التوالي. المستخلصات أيضا أظهرت ارتفاعا بشكل ملحوظ ($P < 0.001$) لتعزيز امتصاص الجلوكوز في الخلايا لمستخلص الإيثانول من *Lagenaria siceraria* الخضار كلها (LSWe)، ومستخلص الإيثانول من *Trichosanthes cucumerina* الخضار كلها (TCWe) ومستخلص المياه من بذور القرع المر (MCSw) تصل إلى 81.31 ، 71.24 و 56.18% على التوالي. تم اختبار تأثير المستخلصات بتركيزات أخرى للاديبونيكيتين وهرمون الليبتين في الخلايا الدهنية باستخدام فحص ال ELISA حيث أشارت إلى ارتفاع ملحوظ ($p < 0.01$) في تركيزات الاديبونيكيتين لمستخلص المياه من القشور (*Lagenaria siceraria* (LSPw)، ومستخلص المياه من موموردিকা القشور *Momordica charantia* (MCPw) ومستخلص المياه من بذور *Trichosanthes cucumerina* (TCSw) بنسب 1557 ± 5405 ، 1097 ± 4966 و 225 ± 3593 نانوجرام / مليلتر على التوالي وانخفاض في تركيزات هرمون الليبتين لمستخلصات LSPw بنسب 612 ± 14633 و 1536 ± 17578 جزء من الجرام / مليلتر على التوالي. وأظهرت بيانات الدراسة أن هناك تأثير مفيد من المستخلصات على تكوين الدهون، تحليل الدهون وامتصاص الجلوكوز في الخلايا الدهنية. وعلاوة على ذلك، قد لوحظ أن تركيز الخلايا الدهنية للاديبونيكيتين والليبتين قد تكون ذات أهمية سريرية في تنظيم الطاقة الذي يشكل عاملا رئيسيا في مرض السكري، والسمنة ومتلازمة التمثيل الغذائي.

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 Health Sciences (Nutrition Sciences).

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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.

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“No pain that we suffer, no trial that we experience is wasted. It ministers to our education, to the development of such qualities as patience, faith, fortitude and humility. All that we suffer and all that we endure, especially when we endure it patiently, builds up our characters, purifies our hearts, expands our souls and it is through sorrow and suffering, toil and tribulation, that we gain the education that we come here to acquire.” O.F.W.

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TABLE OF CONTENTS

Abstract	ii
Approval page	iv
Declaration	v
Declaration	vi
Acknowledgement	vii
Table of Contents	viii
List of Tables	xi
List of Figures	xii
List of abbreviations	xvii
List of Symbols	xviii
List of equations.....	xix
CHAPTER ONE	1
Introduction.....	1
1.1 general introduction	1
1.2 significance of the study.....	3
1.3 objectives.....	4
1.3.1 General objective.....	4
1.3.2 Specific objectives.....	4
1.4 Hypothesis	5
1.5 expected outcome.....	5
CHAPTER TWO	6
Literature Review.....	6
2.1 introduction	6
2.2 general effect of vegetables on health.....	6
2.2.1 Effect on glucose metabolism	7
2.2.2 Effect on lipid metabolism	13
2.2.3 Effect of vegetables on other diseases	15
2.3 provision of nutrients	17
2.3.1 Phytochemicals.....	17
2.3.2 Minerals	19
2.3.3 Vitamins	20
2.3.4 Dietary fibre.....	20
2.4 vegetables used.....	21
2.4.1 Momordica charantia.....	24
2.4.2 Trichosanthes cucumerina	26
2.4.3 Lagenaria siceraria.....	27
2.4.4 Mechanisms of action.....	28
2.4.5 Role of antioxidants.....	30
2.5 cell culture	32
2.5.1 Adipogenesis	33
2.5.2 Adipolysis.....	35

2.5.3 Glucose uptake	36
CHAPTER THREE	37
Materials and methods	37
3.1 Vegetables used.....	37
3.2 General list of chemicals used.....	37
3.3 General list of instruments used.....	38
3.4 Acquisition of cell line	39
3.5 Sample preparation from vegetables.....	39
3.5.1 Extraction of prepared samples	39
3.6 Antioxidant activity.....	41
3.6.1 Determination of total phenol content (TPC).....	41
3.6.2 Determination of total flavonoid content (TFC)	42
3.6.3 Determination of free radical scavenging activity (DPPH).....	42
3.7 Cell culture protocols	43
3.7.1 Cell maintenance and counting	43
3.7.2 Cell viability and proliferation assay.....	44
3.7.3 Differentiation	44
3.7.4 Oil Red O staining	45
3.7.5 Adipolysis.....	46
3.7.6 Glucose uptake	47
3.7.7 Adiponectin and leptin assay.....	47
3.7.8 Statistical analysis	48
CHAPTER FOUR	49
Results and Findings	49
4.1 vegetables extraction.....	49
4.2 Antioxidant activity.....	50
4.2.1 Determination of total phenolic contents (TPC)	50
4.2.2 Determination of total flavonoid contents (TFC).....	53
4.2.3 Determination of free radical scavenging activity (DPPH).....	56
4.3 Bioactivity assay	59
4.3.1 Cell culture protocols	59
CHAPTER FIVE	85
Discussion	85
5.1 Effect of solvents on the extracts	86
5.2 Antioxidant contents	87
5.2.1 Total phenolic contents.....	87
5.2.2 Total flavonoid contents	89
5.2.3 Free radical scavenging activity (DPPH)	91
5.2.4 Relationship between the antioxidant contents and activities ..	93
5.2 Bioactivity assays	99
5.2.5 Effect on cell viability	99
5.2.6 Effect on adipogenesis (lipid synthetic activity)	100
5.2.7 Effect on adipolysis (lipolytic/lipid breakdown activity)	103
5.2.8 Effect on glucose uptake	105
5.2.9 Effect on adiponectin in adipocytes	107
5.2.10 Effects on leptin in adipocytes	110

Conclusion	114
6.1 General conclusion.....	114
6.2 Recommendations for future work	115
References.....	116
Appendix A.....	131
Appendix B	135

LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
2.1	Different names of the vegetables used in the study	26
2.2	total extracts yields of the vegetables using water and ethanol solvents	50
4.3	Summary of the results from various activities of <i>Momordica charantia</i>	91
4.4	Summary of the results from various activities of <i>Trichosanthes cucumerina</i>	92
4.5	Summary of the results from various activities of <i>Lagenaria siceraria</i>	93

LIST OF FIGURES

2.1	Pathophysiology of diabetes: Insulin secretion from the pancreas reduces glucose output by the liver, enhances glucose uptake by skeletal muscle and also suppresses free fatty acid release from adipose tissue. Various factors contribute to the pathogenesis of type 2 diabetes and its dysfunctions which can affect both insulin secretion and insulin action. Decreased insulin secretion will result in a reduction in insulin signaling in the targeted tissues. Source: Stumvoll et al., (2010).	11
2.2	Pharmacological management of hyperglycaemia according to the drugs mechanisms of Source Stumvoll et al., (2010).	14
2.3	Classification of phytochemicals. Source Chen & Blumberg (2008).	20
2.4	The role of antidiabetic plants in improving insulin sensitivity. Source: Eddouks, Bidi, El Bouhali, Hajji, & Zeggwagh (2014).	32
2.5	The main stages of adipogenesis: adipose cells are derived from pluripotent mesenchymal stem cells which undergo a process of cell commitment resulting in preadipocytes which undergo mitotic clonal expansion then differentiation to mature into a full functional adipocyte. Source: Romao, Jin, Dodson, Hausman, & Moore (2015).	36
3.6	Water and solvent extraction methods.	42
4.7	The total phenolic contents of <i>Momordica charantia</i> water and ethanol extracts expressed as mg Gallic acid Equivalent/g.	53
4.8	The total phenolic contents of <i>Trichosanthes cucumerina</i> water and ethanol extracts expressed as mg Gallic acid Equivalent/g.	54
4.9	The total phenolic contents of <i>Lagenaria siceraria</i> water and ethanol extracts expressed as mg Gallic acid Equivalent/g.	54
4.10	The total flavonoid contents of <i>Momordica charantia</i> water and ethanol extracts expressed as mg Quercetin Equivalent/g.	56
4.11	The total flavonoid contents of <i>Trichosanthes cucumerina</i> water and ethanol extracts expressed as mg Quercetin Equivalent/g.	57

4.12	The total flavonoid contents of <i>Lagenaria siceraria</i> water and ethanol extracts expressed as mg Quercetin Equivalent/g.	57
4.13	Percentage inhibition of DPPH radical by <i>Momordica charantia</i> water and ethanol extracts.	60
4.14	Percentage inhibition of DPPH radical by <i>Trichosanthes cucumerina</i> water and ethanol extracts.	61
4.15	Percentage inhibition of DPPH radical by <i>Lagenaria siceraria</i> water and ethanol extracts.	62
4.16	Growth curve of 3T3-L1 preadipocytes. Cells were seeded at a density of 2×10^5 cells/ml and counted daily for eight consecutive days. The maximum growth of 3T3-L1 cells was observed at day 4.	62
4.17	Effect of different concentrations of <i>Momordica Charantia</i> water and ethanol extracts on the proliferation of 3T3-L1 adipocytes. The cells were incubated in the presence of the extracts at decreasing concentrations ranging from 0.031 to 1 mg/ml and the cell viability was evaluated with the MTT assay after 48 hours.	65
4.18	Effect of different concentrations of <i>Trichosanthes cucumerina</i> water and ethanol extracts on the proliferation of 3T3-L1 adipocytes. The cells were incubated in the presence of the extracts at decreasing concentrations ranging from 0.031 to 1 mg/ml and the cell viability was evaluated with the MTT assay after 48 hours.	66
4.19	Effect of different concentrations of <i>Lagenaria siceraria</i> water and ethanol extracts on the proliferation of 3T3-L1 adipocytes. The cells were incubated in the presence of the extracts at decreasing concentrations ranging from 0.031 to 1 mg/ml and the cell viability was evaluated with the MTT assay after 48 hours.	67
4.20	Undifferentiated 3T3-L1 preadipocytes used as a negative control (Mag. 100x).	68
4.21	MDI differentiated adipocytes. (A): differentiated 3T3-L1 adipocytes stained with Oil Red O dye used as a positive control (Mag. 100x). (B): Mature adipocyte stained with Oil Red O (Mag. 200x).	68
4.22	Differentiated 3T3-L1 adipocytes treated with water extract of <i>Momordica charantia</i> whole vegetable (Mag. 100x).	69
4.23	Figure 4.23 Differentiated 3T3-L1 adipocytes treated with water extract of <i>Momordica charantia</i> peels (Mag. 100x).	69

4.24	Differentiated 3T3-L1 adipocytes treated with water extract of <i>Momordica charantia</i> seeds (Mag. 100x).	69
4.25	Differentiated 3T3L1 adipocytes treated with water extract of <i>Trichosanthes cucumerina</i> whole vegetable (Mag. 100x).	70
4.26	Differentiated 3T3L1 adipocytes treated with water extract of <i>Trichosanthes cucumerina</i> peels (Mag. 100x).	70
4.27	Differentiated 3T3L1 adipocytes treated with water extract of <i>Trichosanthes cucumerina</i> seeds (Mag. 100x).	70
4.28	Differentiated 3T3-L1 adipocytes treated with water extract of <i>Lagenaria siceraria</i> whole vegetable (Mag. 100x).	71
4.29	Differentiated 3T3-L1 adipocytes treated with water extract of <i>Lagenaria siceraria</i> peels (Mag. 100x).	71
4.30	Differentiated 3T3-L1 adipocytes treated with water extract of <i>Lagenaria siceraria</i> seeds (Mag. 100x).	71
4.31	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Momordica charantia</i> whole vegetable (Mag. 100x).	72
4.32	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Momordica charantia</i> peels (Mag. 100x).	72
4.33	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Momordica charantia</i> seeds (Mag. 100x).	72
4.34	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Trichosanthes cucumerina</i> whole vegetable (Mag. 100x).	73
4.35	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Trichosanthes cucumerina</i> peels (Mag. 100x).	73
4.36	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Trichosanthes cucumerina</i> seeds (Mag. 100x).	73
4.37	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Lagenaria siceraria</i> whole vegetable (Mag. 100x).	74
4.38	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Lagenaria siceraria</i> peels (Mag. 100x).	74
4.39	Differentiated 3T3-L1 adipocytes treated with ethanol extract of <i>Lagenaria siceraria</i> seeds (Mag. 100x).	74
4.40	The effect of <i>Momordica charantia</i> water and ethanol extracts on 3T3-L1 cell differentiation.	77

4.41	The effect of <i>Trichosanthes cucumerina</i> water and ethanol extracts on 3T3-L1 cell differentiation.	77
4.42	The effect of <i>Lagenaria siceraria</i> water and ethanol extracts on 3T3-L1 cell differentiation.	78
4.43	The effect of <i>Momordica charantia</i> water and ethanol extracts on glycerol release in 3T3-L1 adipocytes.	80
4.44	The effect of <i>Trichosanthes cucumerina</i> water and ethanol extracts on glycerol release in 3T3-L1 adipocytes.	81
4.45	The effect of <i>Lagenaria siceraria</i> water and ethanol extracts on glycerol release in 3T3-L1 adipocytes.	81
4.46	Effect of <i>Momordica charantia</i> water and ethanol extracts on glucose uptake in 3T3-L1 adipocytes.	83
4.47	Effect of <i>Trichosanthes cucumerina</i> water and ethanol extracts on glucose uptake in 3T3-L1 adipocytes.	84
4.48	Effect of <i>Lagenaria siceraria</i> water and ethanol extracts on glucose uptake in 3T3-L1 adipocytes.	84
4.49	Effect of <i>Momordica charantia</i> water and ethanol extracts on adiponectin concentrations in 3T3-L1 adipocytes.	86
4.50	Effect of <i>Trichosanthes cucumerina</i> water and ethanol extracts on adiponectin concentrations in 3T3-L1 adipocytes.	87
4.51	Effect of <i>Lagenaria siceraria</i> water and ethanol extracts on adiponectin concentrations in 3T3-L1 adipocytes.	87
4.52	Effect of <i>Momordica charantia</i> water and ethanol extracts on leptin concentrations in 3T3-L1 adipocytes.	89
4.53	Effect of <i>Trichosanthes cucumerina</i> water and ethanol extracts on leptin concentrations in 3T3-L1 adipocytes.	89
4.54	Effect of <i>Lagenaria siceraria</i> water and ethanol extracts on leptin concentrations in 3T3-L1 adipocytes.	90
5.55	Correlation analysis between phenolic contents and DPPH scavenging activity of <i>Momordica charantia</i> extracts.	103
5.56	Correlation analysis between flavonoid contents and DPPH scavenging activity of <i>Momordica charantia</i> extracts.	103
5.57	Correlation analysis between phenolic contents and DPPH scavenging activity of <i>Trichosanthes cucumerina</i> extracts.	105

5.58	Correlation analysis between flavonoid contents and DPPH scavenging activity of <i>Trichosanthes cucumerina</i> extracts.	105
5.59	Correlation analysis between phenolic contents and DPPH scavenging activity of <i>Lagenaria siceraria</i> extracts.	107
5.60	Correlation analysis between flavonoid contents and DPPH scavenging activity of <i>Lagenaria siceraria</i> extracts.	107

LIST OF ABBREVIATIONS

AMPK	5' adenosine monophosphate-activated protein kinase
cAMP	cyclic Adenosine Monophosphate
ddH ₂ O	double-distilled water
DEX	dexamethasone
DMSO	Dimethylsulfoxide
DMEM	Dulbeco's Modified Eagle Media
DPPH	2,2-diphenyl-1-picrylhydrazyl
EBP	enhancer-binding proteins
et al	(<i>et alia</i>); and others
FBS	Foetal Bovine Serum
GAE	gallic acid equivalent
GLUT	glucose transporters
IBMX	3-isobutyl-1-methylxanthine
i.e.	that is
IUM	International Islamic University Malaysia
KRPH	Krebs-Ringer-Phosphate-HEPES
LSPw	water extract of Lagenaria siceraria peels
LSPe	ethanol extract of Lagenaria siceraria peels
LSSw	water extract of Lagenaria siceraria seeds
LSSe	ethanol extract of Lagenaria siceraria seeds
LSWe	water extract of Lagenaria siceraria whole vegetable
LSWw	water extract of Lagenaria siceraria whole vegetable
MBq	mega becquerel
MCPe	ethanol extract of Momordica charantia peels
MCPw	water extract of Momordica charantia peels
MCSe	Ethanol extract of Momordica charantia seeds
MCSw	water extract of Momordica charantia seeds
MCWe	ethanol extract of Momordica charantia whole vegetable
MCWw	water extract of Momordica charantia whole vegetable
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
PBS	Phosphate Buffered Serum
PI3K	Phosphatidylinositol-3 kinase
PKA	Protein Kinase A
PPAR	peroxisome proliferator-activated receptors
QE	Quercetin equivalent
TCPe	ethanol extract of Trichosanthes cucumerina peels
TCPw	water extract of Trichosanthes cucumerina peels
TCSe	ethanol extract of Trichosanthes cucumerina seeds
TCSw	water extract of Trichosanthes cucumerina seeds
TCWe	ethanol extract of Trichosanthes cucumerina whole vegetable
TCWw	water extract of Trichosanthes cucumerina whole vegetable

LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
Bq	Becquerel
Ci	Curie
g	Gram
mg	Milligram
mmol	Millimolar
ng	Nanogram
pg	Picogram
r	Pearson's correlation
μCi	Micro Curie
mCi	Mili Curie
μg	Microgram
μl	Microliter
$^{\circ}\text{C}$	Degree Celsius
%	Percent
-	To
>	More than
<	Less than
\uparrow	increase
\downarrow	decrease
\pm	Plus-minus
x	Times
=	Equal to
*	Statistical significance denotation

LIST OF EQUATIONS

<u>Equation No.</u>		<u>Page No.</u>
3.1	Determination of free radical scavenging activity (DPPH)	44
3.2	Determination of cell viability	45
3.3	Determination of glycerol concentrations	48
3.4	Determination of cell density and concentration	62

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Vegetables consumption is considered an integral part of daily human diet in all cultures around the world. Fruits and vegetables represent excellent sources for nutrients such as vitamins, minerals, dietary fiber and antioxidants (Kader, 2001). A fruit and vegetable-rich diet has been promoted since the early ages where it has been consumed as whole or segmented such as seeds, leaves, roots, and tubers. Long term consumption of fruits and vegetables may be protective in nature from certain diseases such as cancer (Roleira et al., 2015), cardiovascular disease (Hu, 2011), obesity (Newby, 2009), Diabetes Mellitus (particularly Type 2 Diabetes) (Leisherer, Mündlein, & Drexel, 2013) (Zaklos-Szyda, Majewska, Redzyna, & Koziolkiewicz, 2015), gastrointestinal disorders (Floch & Hong-Curtiss, 2002) and several other diseases. Despite the immense breakthrough in medicine and the wide variety of treatments available in the market, it remains a basic fact that good nutrition is the single cost effective way to improve health and well-being and avoid side effects, the failure of long term desirable results and the economic burden resulting from the use of drugs (Wang, Wang, & Chan, 2013).

The prevalence of non-communicable diseases (NCDs) has been a growing health concern in the recent years around the world including Malaysia. According to World Health Organization (2014), 38 million deaths were recorded worldwide in 2012 that were related to NCDs in which 42% could have been preventable with an adjustment of dietary intakes while diabetes Mellitus prevalence increased from 100 million people in 1990 to 250 million in 2007 and is expected to reach 350 million by

the year of 2030 (NHMS, 2015). Among the Southeast Asian countries, Malaysia is on the top of the charts in the prevalence of Diabetes which increased from 2% in 1960 to 15.2% in 2009 and reached 3.2 million cases in 2014 (4th International Diabetes Conference, 2013). Obesity prevalence went from 200 million obese adults in 1995 to 400 million by the year 2005 with 800 million people being overweight. Malaysia represents the highest country in obesity prevalence in Asia with 45.3% of the population being obese (MASO, 2014). Cardiovascular disease represents the leading cause of death with an estimation of 17.3 million deaths per year worldwide which is expected to grow to 23.6 million by the year of 2030 (World Health Organization, 2014). In Malaysia, the mortality due to CVD has increased from 15.7% in 1996 to 25.4% in 2006 (Amiri et al., 2014) which is a consequence to the high obesity rates. Cancer prevalence has reached 32.5 million people worldwide in 2012 with 9.7% for colorectal cancer while in Malaysia it is estimated that 90 000 to 100 000 people are living with cancer wherein colorectal cancer comes in second place after breast cancer (NCSM, 2012).

The use of plant foods and traditional herbal remedies against NCDs has dominated research recently especially for the treatment and prevention of type 2 Diabetes Mellitus (T2DM) due to its fast expansion throughout the world each year. According to Patel et al., (2012), there are over 1200 species claimed to possess antidiabetic properties although most of them are in lack of the scientific validation, this led the recent researchers to focus more on providing scientific substantiation of these plant foods and the health benefits emitting from their phytochemicals. Among these various and widely spread medicinal plants in Asia generally and Malaysia particularly is the Cucurbitaceae family which, despite its abundance and availability, has not been thoroughly investigated for its potential benefits on diseases. The

vegetables from this family, also called gourds, are considered a great dieting food due to their high water and fiber content and are considered a great source of vitamins A, K and C, minerals such as potassium and antioxidants (flavonoids and carotenoids) (Saboo, Thorat, Tapadiya, & Khadabadi, 2013). Minor research has been made on the investigation of these plants' whole and separated parts for their properties in the prevention of NCDs such as T2DM. The studies aiming for the anti-hyperglycemic effect were mainly focused on certain genres than others. As the incidence of NCDs grows higher each year especially in Malaysia, it is wise to consider alternative preventive tools such as dietary management using fruits and vegetables. This approach can reduce the costs of treatment and help in the prevention of various modern diseases such as T2DM.

In this study, three common Malaysian vegetables of the Cucurbitaceae family, namely: *Momordica Charantia* (also known as Bitter gourd, Karela or Peria Katak), *Lagenaria Siceraria* (known as Bottle gourd, Opo squash or Calabash) and *Trichosanthes Cucumerina* (known as Snake gourd or Labu Ular) were investigated for the effect of their water and ethanol extracts (extracted from whole vegetable, peels and seeds) on 3T3-L1 adipocytes differentiation, adipolysis and glucose uptake in addition to adiponectin and leptin concentrations.

1.2 SIGNIFICANCE OF THE STUDY

Vegetables and their components namely peels, seeds and whole vegetable are being consumed all over the world as parts of the diet. As previously mentioned, these vegetables under investigation are important sources of vitamins, minerals, dietary fiber and of course carbohydrates. However, the components from the seeds and peels are yet to be fully determined and their effect on health conditions lack scientific-based evidence.

In this study, we investigated the effect of three extracts (extracted from whole vegetable, peels and seeds) of three common vegetables grown in highlands of Malaysia namely: *Momordica charantia* (also known as bitter gourd, Peria Katak or Karela), *Trichosanthes Cucumerina* (also known as Labu Ular or snake gourd) and *Lagenaria siceraria* (also known as Labu Air or bottle gourd) on 3T3-L1 adipocytes differentiation, adipolysis, glucose uptake in addition to adiponectin and leptin concentrations. This investigation provides a preliminary understanding of the extracts which have an effect on glucose and lipid metabolism as well as the effect of adipokines on energy regulation observed in 3T3-L1 adipocytes.

1.3 OBJECTIVES

1.3.1 General objective

The main objective of this study was to evaluate the effect of water and ethanol extracts from whole vegetables, peels and seeds of three common Malaysian vegetables, namely: *Momordica charantia*, *Trichosanthes Cucumerina* and *Lagenaria siceraria* and on the differentiation, adipolysis and glucose uptake in 3T3-L1 adipocyte model.

1.3.2 Specific objectives

The specific objectives of this study were as follows:

- i. To determine the total phenol content (TPC), total flavonoid content (TFC) and free radical scavenging activity (DPPH) of the extracts.
- ii. To study and determine the effect of the extracts on adipogenesis and adipolysis.

- iii. To evaluate the effect of the extracts on glucose uptake using a radioactive tracer.
- iv. To assess the effect of the extracts on adiponectin and leptin concentrations in the adipocytes.

1.4 HYPOTHESIS

Momordica charantia, *Trichosanthes Cucumerina* and *Lagenaria siceraria* extracts extracted from whole vegetable, peels and seeds will have the following effects:

- i. antioxidant activity using TPC, TFC and DPPH scavenging activity.
- ii. stimulation of 3T3-L1 differentiation in the adipocytes.
- iii. increase of glycerol release during adipolysis and increase glucose uptake in the adipocytes.
- iv. increase adiponectin concentrations and decrease leptin concentrations in the adipocytes.

1.5 EXPECTED OUTCOME

The treatment of 3T3-L1 adipocytes with the extracts of whole vegetable, peel and seeds of *Momordica charantia*, *Trichosanthes Cucumerina* and *Lagenaria siceraria* will improve the cells' differentiation and adipolysis, increase the glucose uptake in the cells, increase adiponectin concentrations and decrease leptin concentrations in adipocytes. These mechanisms related to adipocytes differentiation, adipolysis and glucose uptake have been linked to a better regulation of glucose and lipid metabolism in the human body as well as the regulation of energy homeostasis especially by the adipokines such as leptin and adiponectin which is beneficial in the prevention of various diseases such as Type 2 Diabetes Mellitus and obesity.