



UNIVERSITI PUTRA MALAYSIA

***DETERMINATION OF ANTI-DIABETIC ACTIVITY OF GYNURA
PROCUMBENS USING BIOASSAY –GUIDED FRACTIONATION***

SITI PAULIENA BINTI MOHD BOHARI

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PROCUMBENS* USING BIOASSAY –GUIDED FRACTIONATION**

By

SITI PAULIENA BINTI MOHD BOHARI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirement for the Degree of Master of Science**

July 2006



DEDICATION

My beloved parents (Mak and Abah)

My sisters and brothers

My Grandfather

Thanks for your prayers, support, encouragement, motivation and sacrifice.....



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

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July 2006

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Faculty : Faculty of Biotechnology and Biomolecular Sciences

This study aims to investigate the antidiabetic and hypoglycaemic properties of Malaysian herb, *Gynura procumbens* (Sambung nyawa). Bioassay guided fractionation has been carried out to identify the bioactive crude fraction responsible for antidiabetic activity of *Gynura procumbens*. Both *in vitro* and *in vivo* model study were used to evaluate the antidiabetic properties of this plant.

In vitro insulin secretion study, glucose uptake study and cytotoxicity were used as primary assay on crude methanolic extract, hexane, ethyl acetate and butanol fractions. Cytotoxicity studies demonstrated that crude methanolic extract have the lowest cytotoxicity when compared with crude fractions *Gynura procumbens* in BRIN BD11 cell lines. Cytotoxicity study with adipocytes and muscle cell lines showed that the crude methanolic extract of *Gynura procumbens* have the lowest toxicity when



compared with the crude fractions of the plant. Determination of insulin secretion response was done by using BRIN BD11 cell lines and from the result, it showed that crude hexane and ethyl acetate crude fractions have good potential in stimulation of insulin release. Glucose uptake study with adipocytes cell lines (3T3 mouse adipocytes cell lines) indicated that this plant has the dose dependent manner and *Gynura procumbens* crude hexane fraction indicated the highest activity on stimulating glucose uptake. Effect of crude methanolic extract and crude fractions in the presence of insulin showed moderate glucose uptake activity when compared with *Gynura procumbens* crude extract/ fractions alone. Glucose uptake study with a mouse L6 muscle cell lines indicated that *Gynura procumbens* crude methanolic extract has highest reading from all of the crude extracts. When comparing the crude extract and fractions with insulin, all of the results showed moderate glucose uptake activity and thus expressed that this plant has dose dependent manner.

Further study was done with Type 1 and Type 2 model diabetic rats. It shows that crude methanolic extract of *Gynura procumbens* have hypoglycemic activity on both models. This result was further investigated with gut perfusion study using crude methanolic extract and ethyl acetate fraction of *Gynura procumbens* and showed positive result by delaying glucose absorption in the rat intestine.

Further investigation was done with ethyl acetate fraction that showed potential activity from *In vitro* and *In vivo* study. Identification of compounds by using reverse phase HPLC showed some promising peaks of interesting compounds. Isolation and

purification was carried on by using various chromatography techniques such as normal chromatography and gel filtration Sephadex LH-20. Two compounds from isolated from ethyl acetate fraction were mix of β -sitosterol and stigmasterol, and kaemferol-3-O- glucoside.

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

**PENGENALPASTIAN AKTIVITI ANTI-DIABETES *GYNURA PROCUMBENS*
MENGUNAKAN BIOASAI BERDASARKAN FRAKSINASI**

Oleh

SITI PAULIENA BINTI MOHD BOHARI

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Kajian ini bertujuan untuk mengkaji ciri-ciri antidiabetes dan hipoglisemik daripada pokok herba Malaysia iaitu *Gynura procumbens* (Sambung nyawa). Teknik pengasingan biocerakinan berpanduan telah digunakan untuk mendapatkan sebatian daripada tumbuhan ini. Untuk menilai ciri-ciri antidiabetes daripada tumbuhan ini, model pegkajian secara *in vivo* dan *in vitro* telah digunakan.

Penyelidikan secara *in vitro* menggunakan asai perembesan insulin, asai pengambilan glukosa dan asai sitotoksik sebagai penaksiran awal bagi ekstrak kasar metanol, fraksi heksana, fraksi etil asetat dan fraksi butanol. Penyelidikan sitotoksik menunjukkan bahawa ekstrak kasar metanol menunjukkan ketoksikan terendah apabila dibandingkan dengan fraksi-fraksi yang lain dalam jujukan sel BRIN BD11. Penyelidikan ketoksikan dengan menggunakan jujukan sel adipos and sel otot menunjukkan bahawa ekstrak metanol *Gynura procumbens* menunjukkan toksik terendah. Penentuan respon perembesan insulin telah dijalankan dengan menggunakan jujukan sel BRIN BD11 dan



keputusan menunjukkan bahawa fraksi heksana dan etil asetat menunjukkan potensi yang bagus dalam mengaruh rembesan insulin apabila dibandingkan dengan kawalan. Penyelidikan pengambilan glukosa dengan jujukan sel adipos (sel 3T3 adipos tikus) menandakan pokok ini memiliki ciri kebergantungan kepada dos ekstrak atau fraksi itu sahaja. Fraksi heksana daripada *Gynura procumbens* memberikan aktiviti tertinggi dalam pengaruh pengambilan glukosa apabila dibandingkan dengan kawalan (tanpa rawatan). Kesan pada ekstrak metanol dan semua fraksi dengan kehadiran insulin hanya menunjukkan aktiviti yang sederhana jika hendak dibandingkan dengan ekstrak dan fraksi-fraksi *Gynura procumbens* sahaja. Penyelidikan pengambilan glukosa dengan sel L6 otot tikus menunjukkan ekstrak metanol *Gynura procumbens* mengaruh pengambilan glukosa dan menunjukkan bacaan tertinggi apabila dibandingkan dengan ekstrak metanol dan semua fraksi tumbuhan ini. Apabila dibandingkan ekstrak bersama insulin, hanya menunjukkan aktiviti yang sederhana dan menunjukan bahawa pokok ini memiliki ciri kebergantungan kepada dos dari tumbuhan itu sahaja.

Kajian diteruskan dengan model tikus diabetes. Kajian menunjukkan bahawa ekstrak metanol *Gynura procumbens* mempunyai aktiviti hipoglisemik terhadap kedua-dua jenis model (Diabetes kategori 1 dan Diabetes kategori 2). Penyelidikan ini diikuti dengan ‘Gut perfusion’ eksperimen yang menggunakan fraksi etil asetat sahaja. Daripada keputusan yang diperolehi menunjukkan keputusan yang memberangsangkan dengan melambatkan penyerapan glukosa ke dalam usus kecil tikus.

Penyelidikan diteruskan lagi dengan fraksi etil asetat yang telah menunjukkan aktiviti yang memberangsangkan selepas diuji dengan ‘*In vitro study*’ dan ‘*In vivo study*’.

Pengenalpastian sebatian dengan menggunakan HPLC 'reverse phase' telah menunjukkan beberapa kemuncak sebatian yang menarik. Pengasingan dan penulenan dilakukan dengan menggunakan pelbagai jenis teknik kromatografi seperti kromatografi normal dan filtrasi gel sephadex LH-20. Dua sebatian telah berjaya diasingkan iaitu campuran β -sitosterol dan stigmasterol dan kaempferol-3-O- glucoside .



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I certify that an Examination Committee has met on 17 July 2006 to conduct the final examination of Siti Pauliena Binti Mohd Bohari on her Master of Science thesis entitled “Determination of Anti-Diabetic Activity of *Gynura procumbens* using Bioassay-Guided Fractionation” accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SITI PAULIENA BINTI MOHD BOHARI

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Diabetes Mellitus	5
2.2 The Endocrine Pancreas	9
2.2.1 Insulin	10
2.2.2 Insulin secretion in β -cell	10
2.2.3 Mechanism of Insulin Action	14
2.2.4 Effect of Insulin on Glucose Uptake	16
2.3 Oral Antidiabetic Drugs	18
2.3.1 Sulfonylureas	19
2.3.2 Biguanides	19
2.3.3 Thiazolidinedions	22
2.3.4 Alpha-Glucosidase Inhibitor	23
2.3.5 Benzoic acid derivatives	25
2.3.6 Glucagon-like peptide 1	26
2.4 Medicinal Plant as antidiabetic agent	26
2.4.1 <i>Gynura procumbens</i>	28
2.4.2 Phytochemical studies	28
2.4.3 Biological activity studies	30
3 MATERIAL AND METHOD	32
3.1 Plant samples	32
3.2 Sample preparation and extraction	32
3.3 Experimental design	
3.3.1 <i>In vitro</i> study	34
3.3.2 <i>In vivo</i> study	42
3.4 Chromatography	46
3.4.1 Isolation of compounds from the ethyl acetate fraction	47
3.5 Spectroscopic Instrumentation	48



3.6	Statistical analysis	50
4	RESULT AND DISCUSSION	
4.1	<i>In vitro</i> study	
4.1.1	Cytotoxicity study	49
4.1.2	Insulin secretion responses on <i>Gynura procumbens</i>	85
4.1.3	Glucose uptake stimulatory	92
4.2	<i>In vivo</i> study	
4.2.1	Acute effect on postprandial glucose level in normal and Type 1 diabetes rat	108
4.2.2	Acute effect on fasting and postprandial glucose level in Type 2 diabetes rat	110
4.2.3	Intestinal glucose absorption	111
4.3	Isolation of compounds from ethyl acetate fraction	119
4.3.1	Structure elucidation of β -sitosterol and Stigmasterol	120
4.3.2	Structure elucidation of Kaempferol-3-O-Glucoside	120
5	CONCLUSION	131
	REFERENCES	136
	APPENDICES	153
	BIODATA OF THE AUTHOR	157
	LIST OF PUBLICATIONS	158

LIST OF TABLES

Table		Page
1	Inhibition concentration 50% (IC ₅₀) on pancreatic beta cell lines towards cytotoxicity study	52
2	Cytotoxicity study methanolic extract of <i>Gynura procumbens</i> in BRIN BD 11 cell lines	54
3	Cytotoxicity study hexane fraction of <i>Gynura procumbens</i> in BRIN BD 11 cell lines	56
4	Cytotoxicity study ethyl acetate fraction of <i>Gynura procumbens</i> in BRIN BD 11 cell lines	58
5	Cytotoxicity study butanol fraction of <i>Gynura procumbens</i> in BRIN BD 11 cell lines	60
6	Cytotoxicity study of glibenclamide in BRIN BD 11 cell lines	62
7	Inhibition concentration 50% (IC ₅₀) on adipocytes cell lines towards cytotoxicity study	63
8	Cytotoxicity study methanolic extract of <i>Gynura procumbens</i> in 3T3 adipocytes cell lines	65
9	Cytotoxicity study hexane fraction of <i>Gynura procumbens</i> in 3T3 adipocytes cell lines	67
10	Cytotoxicity study ethyl acetate extract of <i>Gynura procumbens</i> in 3T3 adipocytes cell lines	69
11	Cytotoxicity study butanol extract of <i>Gynura procumbens</i> in 3T3 adipocytes cell lines	71
12	Cytotoxicity study of metformin in 3T3 adipocytes cell lines	73



13	Inhibition concentration 50% (IC ₅₀) on muscle cell lines towards cytotoxicity study	74
14	Cytotoxicity study methanol extract of <i>Gynura procumbens</i> in L6 muscle cell lines	76
15	Cytotoxicity study hexane extract of <i>Gynura procumbens</i> in L6 muscle lines	78
16	Cytotoxicity study ethyl acetate extract of <i>Gynura procumbens</i> in L6 muscle cell lines	80
17	Cytotoxicity study butanol extract of <i>Gynura procumbens</i> in L6 muscle cell lines	82
18	Cytotoxicity study of Metformin in L6 muscle cell lines	84
19	Insulin secretion activity on <i>Gynura procumbens</i>	86
20	Glucose Uptake activity in adipocytes cell lines	94
21	Glucose Uptake activity in muscle cell lines	102
22	Effect of methanol extract of <i>Gynura procumbens</i> on 30 minutes before blood glucose level of nondiabetic and Type 1 diabetic model rats	109
23	Effect of methanol extract of <i>Gynura procumbens</i> on fasting serum glucose level of nondiabetic and Type 2 diabetic rats	112
24	Effect of methanol extract of <i>Gynura procumbens</i> on simultaneous blood glucose level of nondiabetic and Type 2 diabetic model rats	113
25	Effect of methanol extract of <i>Gynura procumbens</i> on 30 minutes before blood glucose level of nondiabetic and Type 2 diabetic model rats	114
26	The assignment of protons and carbons of kaempferol-3-O-glucoside	130

LIST OF FIGURES

Figure		Page
1	Human Insulin	11
2	The Ionic Control from human insulin pancreatic beta cells	13
3	The insulin receptor and insulin action	15
4	Mechanism of Glucose Uptake	17
5	Structure of Sulphonylureas	20
6	Structure of Biguanides drugs	21
7	Structure of Acarbose	24
8	<i>Gynura procumbens</i>	29
9	Flowchart of extraction and fractionation of <i>Gynura procumbens</i>	33
10	Morphology of BRIN BD 11 cells as assessed by phase contrast microscopy (x200 magnification)	35
11	Morphology of 3T3 F442A adipocytes cells as assessed by phase contrast microscopy (x200 magnification)	36
12	Morphology of L6 muscle cells as assessed by phase contrast microscopy (x200 magnification)	37



13	Cytotoxicity effect of crude methanolic extract <i>Gynura procumbens</i> in BRIN BD 11 cell lines	53
14	Cytotoxicity effect of crude hexane fraction <i>Gynura procumbens</i> in BRIN BD 11 cell lines	55
15	Cytotoxicity effect of crude ethyl acetate fraction <i>Gynura procumbens</i> in BRIN BD 11 cell lines	57
16	Cytotoxicity effect of crude butanol fraction <i>Gynura procumbens</i> in BRIN BD 11 cell lines	59
17	Cytotoxicity effect of glibenclamide in BRIN BD11 cell lines	61
18	Cytotoxicity effect of crude methanolic extract <i>Gynura procumbens</i> in adipocytes cell lines	64
19	Cytotoxicity effect of crude hexane fraction <i>Gynura procumbens</i> in adipocytes cell lines	66
20	Cytotoxicity effect of crude ethyl acetate fraction <i>Gynura procumbens</i> in adipocytes cell lines	68
21	Cytotoxicity effect of crude butanol fraction <i>Gynura procumbens</i> in adipocytes cell lines	70
22	Cytotoxicity effect of metformin in adipocytes cell lines	72
23	Cytotoxicity effect of crude methanolic extract <i>Gynura procumbens</i> in muscle cell lines	75
24	Cytotoxicity effect of crude hexane fraction <i>Gynura procumbens</i> in muscle cell lines	77



25	Cytotoxicity effect of crude ethyl acetate fraction <i>Gynura procumbens</i> in muscle cell lines	79
26	Cytotoxicity effect of crude butanol fraction <i>Gynura procumbens</i> in muscle cell lines	81
27	Cytotoxicity effect of metformin in adipocytes cell lines	83
28	Effect of crude methanolic extract <i>Gynura procumbens</i> on insulin secretion by BRIN BD 11 cell lines after 30 minutes incubation	87
29	Effect of crude hexane extract <i>Gynura procumbens</i> on insulin secretion by BRIN BD 11 cell lines after 30 minutes incubation	88
30	Effect of crude ethyl acetate extract <i>Gynura procumbens</i> on insulin secretion by BRIN BD 11 cell lines after 30 minutes incubation	89
31	Effect of crude butanol extract <i>Gynura procumbens</i> on insulin secretion by BRIN BD 11 cell lines after 30 minutes incubation	90
32	Effect of Glibenclamide <i>Gynura procumbens</i> on insulin secretion by BRIN BD 11 cell lines after 30 minutes incubation	91
33	Effect of the various concentration of <i>Gynura procumbens</i> in methanolic extract on glucose uptake in adipocytes cell lines	95
34	Effect of the various concentration of <i>Gynura procumbens</i> in hexane crude fraction on glucose uptake in adipocytes cell lines	96
35	Effect of the various concentration of <i>Gynura procumbens</i> in ethyl acetate crude fraction on glucose uptake in adipocytes cell lines	97
36	Effect of the various concentration of <i>Gynura procumbens</i> in butanol crude fraction on glucose uptake in adipocytes cell lines	98



37	Effect of metformin on glucose uptake in adipocytes cell lines	99
38	Effect of the various concentration of <i>Gynura procumbens</i> in methanolic extract on glucose uptake in muscle cell lines	103
39	Effect of the various concentration of <i>Gynura procumbens</i> in hexane crude fraction on glucose uptake in muscle cell lines	104
40	Effect of the various concentration of <i>Gynura procumbens</i> in ethyl acetate crude fraction on glucose uptake in muscle cell lines	105
41	Effect of the various concentration of <i>Gynura procumbens</i> in butanol crude fraction on glucose uptake in muscle cell lines	106
42	Effect of the metformin on glucose uptake in muscle cell	107
43	Effect of methanolic extract of <i>Gynura procumbens</i> in glucose absorption in the gut	116
44	Effect of ethyl acetate fraction of <i>Gynura procumbens</i> in glucose absorption in the gut	117
45	Glucose absorption in the gut with ouabain	118
46	HPLC analysis of ethyl acetate fraction of <i>Gynura procumbens</i>	122
47	Structure of mix β -sitosterol and stigmasterol	123
48	HNMR spectrum of mix β -sitosterol and stigmasterol	124
49	Mass spectrum of mix β -sitosterol and stigmasterol	125
50	HNMR spectrum of kaempferol-3-O-glucoside	126



51	CNMR spectrum of kaempferol-3-O-glucoside	127
52	Cosy spectrum of kaempferol-3-O-glucoside	128
53	Structure of kaempferol-3-O-glucoside	129



LIST OF ABBREVIATIONS

β	Beta
BIRDEM	Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorder
BSA	Bovine serum albumin
b.w	Body weight
^{13}C	Carbon 13
CO_2	Carbon dioxide
cc	Column chromatography
δ	Chemical shift in ppm
CHCl_3	Chloroform
cm^2	Centimeter square
$^\circ\text{C}$	Degree Celsius
DMSO	Dimethyl sulfoxide
d	Doublet
dd	Doublet of doublet
DEPT	Distortionless Enhancement by polarization Transfer
ELISA	Enzyme-Linked Immunosorbent Assay
EtoAC	Ethyl acetate
FBS	Fetal bovine serum
g	Gram
GDM	Gestational diabetes mellitus



GLUT4	Glucose transporter 4
HMBC	Heteronuclear Multiple Bond Correlation
Hz	Hertz
HPLC	High Performance Layer Chromatography
hr	Hour
IC ₅₀	Concentration of 50% inhibition
IDDM	Insulin dependent diabetes mellitus
Kg	Kilogram
L	Litre
M	Molar
MeOH	Methanol
μl	Microlitre
mg	Miligram
NIDDM	Non insulin diabetes mellitus

CHAPTER 1

INTRODUCTION

Diabetes mellitus is a metabolic disorder in the endocrine system. The disease is one of the major public health concerns and is rapidly increasing in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose.

As a very common chronic disease, diabetes is becoming the third ‘killer’ along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, morbidity and mortality (Li *et al.*, 2004). The cause of diabetes is a mystery, although both genetic and environmental factors such as obesity and lack of exercise appear to play a role. Ethnic and racial differences have been found in heterogenous populations within the same area. Most researchers believe that in the presence of a genetic predisposition, something in the environment triggers the development of the diabetes. With a long cause and serious complications often resulting in high death-rate, the treatment of diabetes spent vast amounts of resources including medicines, diets, and physical training in all countries. Up to now, many kinds of antidiabetic medicines have been developed for patients, but almost all are chemical or biochemical agents. The fact is that there has been no report of someone recovering totally from diabetes (Li *et al.*, 2004).

