



UNIVERSITI PUTRA MALAYSIA

**NUTRITIONAL COMPOSITION OF *STROBILANTHES CRISPUS*
JUICE AND ITS EFFECTS ON HYPERGLYCAEMIA,
HYPERLIPIDEMIA, WOUND HEALING AND TOXICITY IN RATS**

NORFARIZAN HANOON BT NOOR AZMI

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By

NORFARIZAN HANOON BT NOOR AZMI

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Chairperson : Professor Asmah Bt. Rahmat, PhD

Faculty : Medicine and Health Sciences

Strobilanthes crispus juice is reported to have good medicinal properties for treating diabetes mellitus and wound healing. The first part of this study evaluated the effect of *S. crispus* juice on hyperglycaemic, hyperlipidemic and antioxidant enzymes in normal and STZ-induced hyperglycaemic male and female rats at dosages of 140, 210 and 280 mg/kg of body weight (bw) for 30 days. Serum glucose, lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol), antioxidant enzymes (glutathione peroxidase and superoxide dismutase) were determined on day 0, day 15 and day 30. The result showed that significant decrease of serum glucose levels in male and female diabetic rats with treated glibenclamide and all groups treated with 140, 210 and 280 mg/kg bw of *S. crispus* juice on days 15 and 30 when compared to control group and baseline data (zero day). The highest reduction of glucose level was 80.9 % on day 30 in male diabetic group treated with 280 mg/kg bw *S. crispus* juice, followed by 78.9 % reduction in group treated with 210 mg/kg bw, and 67.4 % reduction in group treated with 140 mg/kg bw of *S. crispus* juice. In

female diabetic groups, reduction of glucose level was 78.2 %, 68.9 % and 68.6 % in groups treated with 140, 210 and 280 mg/kg bw of *S. crispus* juice respectively. Administration of *S. crispus* juice also reduced total cholesterol, triglyceride, LDL-cholesterol; increased HDL-cholesterol, the activity of glutathione peroxidase and superoxide dismutase in STZ-induced diabetic and normal rats.

Second part of this study was to determine the effect of *S. crispus* juice on wound healing and antioxidant enzymes (glutathione peroxidase and superoxide dismutase) in normal and diabetic rats. Three levels of dosage (70, 100 and 140 mg/kg of body weight) were orally and repeatedly administered for 7 days. Mid-dorsal linear incisions of 2 cm in length were made on each animal. The results showed that supplementation of *S. crispus* juice enhances wound closure in normal and diabetic rats. Glutathione peroxidase and superoxide dismutase were increased in diabetic group treated with *S. crispus* juice.

Third part of this study investigated the proximate composition, vitamin and mineral contents of *Strobilanthes crispus* juice. The proximate analysis showed that *S. crispus* juice contained high moisture (75.01 %), carbohydrate content (33.47 %) and dietary fibre (12.29 g/100g). The contents of vitamin A, C and E in *S. crispus* juice were found to be 2.32 mg/100g, 9.37 mg/100g and 5.89 mg/100g respectively. *S. crispus* juice was found high in minerals including, sodium (37.21 mg/100g), potassium (124.99 mg/100g), calcium (172.88 mg/100g), ferum (0.57 mg/100g), phosphorus (8.18 mg/100g), magnesium (27.86 mg/100g), copper (0.14 mg/100g) and zinc (1.49 mg/100g).

The fourth part of this study evaluated the acute toxicity of *S. crispus* juice. The LD₅₀ was determined with four different dosages of *S. crispus* juice (700, 2100, 3500 and 4900 mg/kg of body weight) administered orally to normal female and male rats. The rats were treated with a single dose of juice and toxic effects were observed within 7 days. The results showed that no death or toxicity signs were observed in LD₅₀ tested in normal rats. In the blood chemistry tests, no significant changes ($p < 0.05$) were observed for most parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and albumin) tested in normal rats. The 30 days toxicity effect were determined by the repeated dosing study using normal and streptozotocin-induced diabetic rats of both sexes. Three level of dosage (140, 210 and 280 mg/kg of body weight) were orally and repeatedly administered for 30 days. The results showed that no significant changes in general behaviour, body weight and organ weight. No differences were noted between the test and control groups in haematological, macroscopic and histopathological findings. The administration of *S. crispus* juice to normal rats revealed insignificant change in liver and kidney functions, but significant reduction of aspartate aminotransferase (group female and male rats with administration 280 mg/kg b.w. of *S. crispus* juice), alanine aminotransferase (group female rats with 280 mg/kg b.w. of *S. crispus* juice) and alkaline phosphatase (group female rats with 210 mg/kg b.w. of *S. crispus* juice). In streptozotocin-induced diabetes, the rise in blood glucose is accompanied by an increase in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatinine. After the administration of diabetic rats with *S. crispus* juice for 30 days, there was a significant reduction in AST, ALT, ALP and creatinine. In conclusion, *S. crispus* juice has high nutritional

value and found non-toxic. It shows potential as an antidiabetic drink and additional nutraceutical supplement for wound healing for diabetic patients.

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

**KOMPOSISI NUTRIEN JUS *STROBILANTHES CRISPUS* DAN KESAN KE
ATAS HIPERGLISEMIA, HIPERLIPIDEMIA, PENYEMBUHAN LUKA
DAN KETOKSIKAN PADA TIKUS**

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Jus *Strobilanthes crispus* mempunyai potensi perubatan yang tinggi di dalam merawat diabetes mellitus dan penyembuhan luka. Pada peringkat pertama kajian ini menilai kesan jus *S. crispus* pada dos 140, 210 dan 280 mg/kg berat badan ke atas hiperglisemik, hiperlipidemik dan enzim antioksidasi bagi tikus jantan dan betina yang normal dan hiperglisemik diaruh streptozotocin selama 30 hari. Serum glukosa, profil lipid (jumlah kolesterol, trigliserida, HDL-kolesterol, LDL-kolesterol), enzim antioksidasi (glutathione peroksida dan superoxide dismutase) ditentukan pada hari 0, 15 dan 30. Keputusan menunjukkan penurunan yang signifikan bagi tahap serum glukosa pada tikus diabetik jantan dan betina yang dirawat dengan glibenclamide dan kesemua kumpulan yang dirawat dengan jus *S. crispus* 140, 210 dan 280 mg/kg berat badan pada hari ke 15 dan 30 dibandingkan dengan kumpulan kawalan dan data asas. Tahap penurunan glukosa yang tertinggi adalah sebanyak 80.9 % bagi kumpulan diabetik tikus jantan yang dirawat jus *S. crispus* pada hari ke 30 dengan dos 280

mg/kg berat badan, diikuti oleh penurunan sebanyak 78.9 % bagi kumpulan yang dirawat 210 mg/kg berat badan dan 67.4 % bagi kumpulan yang dirawat 140 mg/kg berat badan. Bagi kumpulan diabetik betina, penurunan tahap glukosa adalah 78.2 %, 68.9 % dan 68.6 % pada kumpulan yang dirawat jus *S. crispus* 280, 210 dan 140 mg/kg b.w. Pemberian jus *S. crispus* juga dapat menurunkan jumlah kolesterol, trigliserida, LDL-kolesterol; meningkatkan HDL-kolesterol, aktiviti glutathione peroksidase dan superoxide dismutase pada tikus diabetik diaruh STZ dan tikus normal.

Pada peringkat kedua kajian ini adalah menentukan kesan jus *S. crispus* pada penyembuhan luka dan enzim antioksidasi (glutathione peroxidase dan superoxide dismutase) di dalam tikus normal dan diabetik. Tiga tahap dos (70, 105 dan 140 mg/kg berat badan) diberikan oral selama 7 hari. Luka sepanjang 2 cm dilakukan di tengah belakang setiap tikus. Keputusan menunjukkan pemberian jus *S. crispus* mengalakkan penyembuhan luka pada tikus normal dan diabetik. Enzim glutathione peroxidase dan superoxide dismutase menunjukkan peningkatan bagi kumpulan diabetik yang dirawat dengan jus *S. crispus*.

Pada peringkat ketiga kajian ini adalah menentukan komposisi proksimat, kandungan vitamin dan mineral bagi jus *S. crispus*. Analisis proksimat menunjukkan jus *S. crispus* mempunyai kandungan air (75.01 %), karbohidrat (33.47 %) dan gentian diet yang tinggi (12.29 g/100g). Kandungan vitamin A, C dan E yang diperolehi bagi jus *S. crispus* adalah 2.32 mg/100g, 9.37 mg/100g dan 5.89 mg/100g. Jus *S. crispus* mempunyai kandungan mineral yang tinggi iaitu sodium (37.21 mg/100g), potassium (124.99 mg/100g), kalsium (172.88 mg/100g), ferum (0.57 mg/100g), fosforus (8.18

mg/100g), magnesium (27.86 mg/100g), kuprum (0.14 mg/100g) dan zink (1.49 mg/100g).

Pada peringkat keempat kajian ini adalah menilai keselamatan jangka pendek (akut) bagi jus *S. crispus*. Ujian DM_{50} ditentukan dengan menggunakan empat dos jus *S. crispus* yang berlainan (700, 2100, 3500 dan 4900 mg/kg berat badan) yang diberikan secara oral (suapan) kepada tikus betina dan jantan yang normal. Tikus tersebut diberikan jus secara dos tunggal dan sebarang kesan toksik diperhatikan dalam masa 7 hari. Keputusan menunjukkan tiada kematian atau tanda ketoksikan melalui pemerhatian pada ujian DM_{50} pada tikus normal. Keputusan ujian kimia darah menunjukkan tiada perubahan yang signifikan yang dinilai ke atas kebanyakan parameter biokimia (aspartat aminotransferase, alanin aminotransferase, alkalin fosfatase, kreatinin dan albumin) yang diuji pada tikus normal. Pada 30 hari kesan toksikologi yang ditentukan oleh ujian subakut yang menggunakan tikus normal dan diabetik yang disuntik streptozotocin pada kedua-dua jantina. Tiga tahap dos (140, 210 dan 280 mg/kg berat badan) yang diberikan setiap hari selama 30 hari kepada tikus normal dan diabetik. Keputusan yang diperolehi menunjukkan tiada perubahan yang signifikan pada pemerhatian bagi kelakuan, berat badan dan berat organ. Tiada perbezaan didapati di antara kumpulan ujian dengan kawalan bagi keputusan hematologi, makroskopi dan histopatologi. Pemberian jus *S. crispus* kepada tikus normal didapati tiada perbezaan yang signifikan bagi fungsi hati dan ginjal kecuali penurunan yang signifikan bagi aspartat aminotransferase (bagi kumpulan tikus betina dan jantan yang diberikan jus *S. crispus* 280 mg/kg berat badan), alanin aminotransferase (kumpulan tikus betina dengan pemberian jus *S. crispus* 280 mg/kg berat badan), dan alkalin fosfatase (kumpulan tikus betina dengan pemberian jus *S.*

crispus 210 mg/kg berat badan). Pada tikus diabetik diaruh streptozotocin, peningkatan glukosa di dalam darah menyebabkan peningkatan serum aspartat aminotransferase (AST), alanin aminotransferase (ALT), alkalin fosfatase (ALP) dan kreatinin. Selepas rawatan ke atas tikus diabetik dengan jus *S. crispus* selama 30 hari menunjukkan penurunan yang signifikan bagi AST, ALT, ALP dan kreatinin. Kesimpulannya, jus *S. crispus* mempunyai nilai pemakanan yang tinggi yang berpotensi dan tidak toksik. Ia menunjukkan potensi sebagai minuman antidiabetik dan nutraseutikal yang dapat menyembuhkan luka pada pesakit diabetik.

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I certify that a Thesis Examination Committee has met on 25 June 2009 to conduct the final examination of Norfarizan Hanoon Bt Noor Azmi on her thesis entitled “Nutritional Composition of *Strobilanthes crispus* juice and its Effects on Hyperglycaemia, Hyperlipidemia, Wound Healing and Toxicity in Rats” in accordance with the Universities and University Colleges 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 Doctor of Philosophy.

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DECLARATION

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

NORFARIZAN HANOON BT NOOR AZMI

Date: 1 July 2009

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xviii
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
1.1 Background	1
1.2 Problem statement	3
1.3 Objectives of research	5
1.3.1 General objective	5
1.3.2 Specific objectives	5
1.4 Significance of the research	6
2 LITERATURE REVIEW	
2.1 Status of herbal medicine in Malaysia	7
2.2 <i>Strobilanthes crispus</i>	8
2.2.1 <i>Strobilanthes crispus</i> used for medicinal purposes	10
2.3 Diabetes Mellitus	11
2.3.1 Major classification of diabetes mellitus	14
2.3.2 Diagnostic criteria of diabetes mellitus	17
2.3.3 Symptom of diabetes mellitus	18
2.3.4 Complications associated with diabetes mellitus	19
2.3.5 Plasma lipids, cardiovascular disease and diabetes	23
2.3.6 Oxidative stress and diabetes	26
2.3.7 Treatment of diabetes mellitus	35
2.4 Wound and diabetes	41
2.4.1 Normal wound healing	41
2.4.2 Diabetic wound healing	44
2.4.3 Treatment of wound	46
2.5 Nutrient and disease	49
2.5.1 Dietary fibre and diabetes	49
2.5.2 Magnesium and diabetes	50
2.5.3 Vitamin E and diabetes	51
2.5.4 Vitamin E and wound healing	51
2.5.5 Vitamin A and wound healing	52
2.5.6 Vitamin C and wound healing	52
2.5.7 Protein and wound healing	53
2.5.8 Zinc and wound healing	54
2.6 Toxicity study	55

2.6.1	<i>In- vivo</i> toxicity study	55
2.6.2	Liver and liver function test	70
2.6.3	Kidney and renal function test	74
3	EFFECT OF <i>STROBILANTHES CRISPUS</i> JUICE ON HYPERGLYCEMIA, HYPERLIPIDEMIA AND ANTIOXIDANT ENZYMES IN STREPTOZOTOZIN-INDUCED DIABETIC AND NORMAL MALE AND FEMALE RATS	76
3.1	Introduction	76
3.2	Materials and Methods	78
3.2.1	Study protocol	78
3.2.2	Statistical analysis	88
3.3	Results	88
3.3.1	Effect of <i>S. crispus</i> juice on serum glucose level	88
3.3.2	Effect of <i>S. crispus</i> juice on cholesterol level	93
3.3.3	Effect of <i>S. crispus</i> juice on triglyceride level	93
3.3.4	Effect of <i>S. crispus</i> juice on high density lipoprotein-cholesterol level	98
3.3.5	Effect of <i>S. crispus</i> juice on low density lipoprotein-cholesterol level	98
3.3.6	Effect of <i>S. crispus</i> juice on glutathione peroxidase	103
3.3.7	Effect of <i>S. crispus</i> juice on superoxide dismutase	103
3.4	Discussion	109
3.5	Conclusion	112
4	EFFECTS OF <i>STROBILANTHES CRISPUS</i> JUICE ON WOUND HEALING AND ANTIOXIDANT ENZYMES IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS	114
4.1	Introduction	114
4.2	Materials and Methods	116
4.2.1	Experimental rats	116
4.2.2	Induction of experimental diabetes	116
4.2.3	Wound creation	117
4.2.4	Experimental procedure	117
4.2.5	Blood preparation	118
4.2.6	Determination of glutathione peroxidase enzymes	118
4.2.7	Determination of superoxide dismutase enzymes	118
4.2.8	Statistical analysis	119
4.3	Results	120
4.4	Discussion	124
4.5	Conclusion	127
5	NUTRITIONAL COMPOSITION OF <i>STROBILANTHES CRISPUS</i> JUICE	128
5.1	Introduction	128
5.2	Materials and methods	129
5.2.1	Nutrient composition	129
5.2.3	Statistical analysis	140

5.3	Results	140
5.3.1	Proximate composition and antioxidant vitamin	140
5.3.2	Mineral content	141
5.4	Discussion	145
5.5	Conclusion	148
6	ACUTE AND 30-DAY TOXICITY STUDY OF <i>STROBILANTHES CRISPUS</i> JUICE IN FEMALE AND MALE RATS	149
6.1	Introduction	149
6.2	Methodology	150
6.2.1	Experimental rats and study design	151
6.2.2	Study design and dosage	151
6.2.3	Induction of experimental diabetes	153
6.2.4	Observations	153
6.2.5	Gross pathological evaluations	154
6.2.6	Biochemical analysis	154
6.2.7	Determination of liver function test	155
6.2.8	Determination of kidney function test	157
6.2.9	Haematology parameter	158
6.2.10	Histology examination	158
6.2.11	Statistical analysis	161
6.3	Results	161
6.3.1	Acute toxicity study	161
6.3.2	30-day toxicity study	171
6.4	Discussion	201
6.5	Conclusion	204
7	GENERAL DISCUSSIONS	205
8	CONCLUSIONS	212
8.1	General conclusions	212
8.2	Recommendations for future research	213
	BIBLIOGRAPHY	215
	APPENDICES	236
	BIODATA OF STUDENT	241
	LIST OF PUBLICATIONS	242

LIST OF TABLES

Table		Page
2.1	Etiological classification of diabetes	12
2.2	Comparison between type 1 and type 2 diabetes mellitus	14
2.3	Treatment options in diabetes	37
2.4	Changes in diabetic ulcers in patients and in wounds of diabetic animals	45
3.1	Serum glucose level (mmol/l) in male and female STZ- induced diabetic rats	90
3.2	Percentage of glucose reduction in treated male and female STZ- induced diabetic rats at day 15 and day 30	91
3.3	Serum glucose level (mmol/l) in male and female normal rats	92
3.4	Total cholesterol level (mmol/l) in male and female STZ- induced diabetic rats	94
3.5	Total cholesterol level (mmol/l) in male and female normal rats	95
3.6	Triglyceride level (mmol/l) in male and female STZ-induced diabetic rats	96
3.7	Triglyceride level (mmol/l) in male and female normal rats	97
3.8	HDL-cholesterol level (mmol/l) in male and female STZ- induced diabetic rats	99
3.9	HDL-cholesterol level (mmol/l) in male and female normal rats	100
3.10	LDL-cholesterol level (mmol/l) in male and female STZ-induced diabetic rats	101
3.11	LDL-cholesterol level (mmol/l) in male and female normal rats	102
3.12	Glutathione peroxidase activity (u/ml) in male and female diabetic rats.	105
3.13	Glutathione peroxidase activity (u/ml) in male and female normal rats	106
3.14	Superoxide dismutase activity (u/L) in male and female diabetic rats.	107
3.15	Superoxide dismutase activity (u/L) in male and female normal	108

	rats	
4.1	Length of wound (cm) in diabetic and normal group	120
4.2	Percentage of wound closure in diabetic and normal rats	122
4.3	Glutathione peroxidase activity (u/ml) in diabetic and normal group	123
4.4	Superoxide dismutase activity (u/L) in diabetic and normal group	124
5.1	Conditions for HPLC separation	140
5.2	Chemical composition of <i>S. crispus</i> juice, <i>Strobilanthes crispus</i> juice, <i>Strobilanthes crispus</i> tea and <i>Strobilanthes crispus</i> leaf	143
5.3	Minerals in <i>Strobilanthes crispus</i> juice, <i>Strobilanthes crispus</i> tea and <i>Strobilanthes crispus</i> leaf	144
6.1	Steps in tissue dehydration process	159
6.2	Hematoxylin and eosin staining process	160
6.3	Body weight (g) in female and male Sprague-dawley rats	162
6.4	Liver weight and relative liver weight in female and male rats	163
6.5	Kidney weight and relative kidney weight in female and male rats	164
6.6	Aspartate aminotransferase (AST) activity ($\mu\text{kat/l}$) in female and male Sprague-dawley rat	165
6.7	Alanine aminotransferase (ALT) activity ($\mu\text{kat/l}$) in female and male rats	167
6.8	Alkaline phosphatase (ALP) activity (μl) in female and male rats	168
6.9	Serum albumin level (g/l) in female and male Sprague-dawley rat	169
6.10	Creatinine level (mg/dl) in female and male rats	170
6.11	Body weight of rats fed with <i>S. crispus</i> juice for 30 days	172
6.12	Kidney weight (g) and ratio of kidney per body weight in female and male normal rats	173

6.13	Liver weight (g) and ratio of liver per body weight in female and male normal rats	174
6.14	Aspartate aminotransferase (AST) level ($\mu\text{kat/l}$) in female and male normal rats	175
6.15	Alanine aminotransferase (ALT) level ($\mu\text{kat/l}$) in female and male normal rats	176
6.16	Alkaline phosphatase (ALP) level ($\mu\text{kat/l}$) in female and male normal rats	177
6.17	Albumin level (g/dL) in female and male normal rats	178
6.18	Creatinine level (mg/dl) in female and male normal rats	179
6.19	Hematology parameter in female normal rats	180
6.20	Hematology parameter in male normal rats	181
6.21	Body weight (g) in male and female diabetic rats	192
6.22	Relative kidney and liver weight in female and male diabetic rats	193
6.23	Aspartate aminotransferase (AST) level ($\mu\text{kat/l}$) in male and female diabetic rats	194
6.24	Alanine aminotransferase (ALT) level ($\mu\text{kat/l}$) in male and female diabetic rats	195
6.25	Alkaline phosphatase (ALP) level (u/l) in female and male diabetic rats.	196
6.26	Serum albumin level (g/l) in male and female diabetic rat	199
6.27	Creatinine level (mg/dl) in female and male diabetic rats	200

LIST OF FIGURES

Figure		Page
2.1	Pathways of ROS formation , the lipid peroxidation process and the role of glutathione and other antioxidants in management of oxidative stress	30
2.2	Overview of growth factor involvement in normal epidermal wound healing	43
2.3	Stages of wound repair	43
4.1	Photo of wound closure in diabetic rats	121
6.1	Light micrographs of the rat liver in male and female control, and after administration with 1.0, 1.5 and 2.0 ml.kg <i>S. crispus</i> juice	182
6.2	Light micrographs of the rat kidney (cortical part) in male and female control, and after administration with 1.0, 1.5 and 2.0 ml.kg <i>S. crispus</i> juice	187

LIST OF ABBREVIATIONS

ACUC	Animal Care and Use Committee
ADA	American Diabetes Association
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AOAC	Association of Official Analytical Chemists
g	gram
GPx	Glutathione peroxidase
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
H ₂ SO ₄	Sulphuric acid
HDL	High Density Lipoprotein
IMR	Institute of Medical Research
KCl	Potassium chloride
K ₂ SO ₄	Potassium sulphate
LD ₅₀	Median lethal dose
LDL	Low Density Lipoprotein
μkat/L	Microkat per liter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MES-TRIS	Morpholino ethansulfonic acid- TRIS-hydroxy-methyl aminomethane
pg	Pico gram
SCJ	<i>Strobilanthes crispus</i> juice

SOD Superoxide dismutase

WHO World Health Organization



CHAPTER 1

INTRODUCTION

1.1 Background

Herbs refer to non-woody seed-producing plants that are annual, biennial or perennial and die at the end of each growing season. Several parts of the plant such as the flower, stem, seed or root are used for medical or aromatherapy qualities. In general, health care professionals consider herbs to be a crude drug, which is used to prevent diseases, treat infection or maintain a state of health (McCann, 2004). Herbs can be used as extracts, juices, tea, lozenges, inhalation, oils, salves, capsules and herbal baths.

Herbs have found its place in modern day biomedical medications. About one-fourth of all biomedical medications commonly prescribed today, contain at least an active ingredient derived from plants and the additional contents are chemically synthesized in the laboratory (McCann, 2004). Herbs are advertised as being able to improve overall health, cure illness and control diseases from diabetes to AIDS (Magee and Loiacono, 2004).

Herbal medicine are plant derived material or preparations with therapeutic or other human health benefit which contain either raw or processed ingredients from one or more plants. In some traditions material of inorganic or animal origin may also be present (WHO, 2005). Herbal medicine as the major remedy in traditional medical

