

**Isolation and identification of a novel anti-diabetic compound
from *Euclea undulata* Thunb.**

by

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(Department of Plant Science)
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2010



Declaration

I declare that this thesis, which I hereby submit for the degree PhD Plant Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:

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Isolation and identification of a novel anti-diabetic compound from *Euclea undulata* Thunb.

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Abstract

Four plant species traditionally used for the treatment of diabetes by South African traditional healers and herbalists were investigated for hypoglycaemic activity. Species included *Schkuhria pinnata* (Lam.) Cabrera, *Pteronia divaricata* (P.J. Bergius) Less *Elaeodendron transvaalense* (Burt Davy) R.H. Archer and *Euclea undulata* Thunb var. *myrtina* (Burch.) Hiern.

Acetone and ethanol plant extracts were prepared and tested *in vitro*, for glucose utilization, at concentrations of 12.5 µg/ml on three cell lines namely; Murine C2C12 myocytes, Chang liver cells and 3T3-L1 preadipocytes. Metformin, at a concentration of 1 µM (0.166 µg/ml) was used as positive control for hepatic cells and insulin at a concentration 1 µM (5.7 µg/ml) for myocytes and preadipocytes. Toxicity tests were done for all extracts on preadipocytes and hepatic cells, but not on myocytes as these cells were exposed to the extract for only a short period (1 hour) during the hypoglycaemic bioassay. Preadipocytes and hepatic cells were exposed to the plant extracts for 48 hours. The four plant extracts were further investigated for hypoglycaemic activity by evaluating inhibiting effects on carbohydrate-hydrolysing enzymes alpha-glycosidase and alpha-amylase.

In vitro hypoglycaemic analysis revealed that acetone and ethanol plant extracts of *S. pinnata*, *E. undulata* and *E. transvaalense*, displayed hypoglycaemic activity in one or more of the various cell lines, whereas, *P. divaricata* showed no hypoglycaemic activity. The best results were obtained with the ethanol and acetone extracts of *S. pinnata* in

preadipocytes with a glucose uptake of 148.2% and 79.6% respectively, above control (100%). However, about 50% preadipocytes survived on exposure to the extracts of *S. pinnata* at 12.5 µg/ml indicating significant cytotoxicity. Glucose uptake of 63.3% was observed by the ethanol extract of *S. pinnata* on hepatic cells. *E. transvaalense* showed hypoglycaemic activity on preadipocytes exhibiting glucose uptake of 38.6% above control 100%. Glucose uptake of 62.2 % were obtained by the *E. undulata* extract in C2C12 myocytes, with 100% cell viability. *E. undulata* scored a +3 and was chosen for further analysis. Antidiabetic activity and toxicity of the plant extracts were taken into consideration when scoring was applied.

Alpha-glucosidase and alpha-amylase results indicated that *P. divaricata* extract inhibited alpha-glucosidase (IC₅₀ 31.22 µg/ml) whereas *E. undulata* (IC₅₀ 2.80 µg/ml) and *E. transvaalense* (IC₅₀ 1.12 µg/ml) extracts inhibited alpha-amylase. Results obtained indicated that all four plant extracts tested have the ability to lower blood glucose levels to some extent and in different manners and therefore corroborate the ethnomedicinal use of these four species in the treatment of diabetes.

Phytochemical studies of a crude acetone extract of the root bark of *E. undulata* var. *myrtina* produced a new α-amyrin-3O-β-(5-hydroxy) ferulic acid compound (**1**), and three known compounds; betulin (**2**), lupeol (**3**) and epicatechin (**4**). The chemical structures were determined by spectroscopic means. *In vitro* assays on C2C12 myocytes revealed that (**2**) (21.4%) and (**4**) (166.3%) were active in lowering blood glucose levels whereas (**1**) (IC₅₀ 4.79 µg/ml) and to a lesser extent (**4**) (IC₅₀ 5.86 µg/ml) and (**3**) (IC₅₀ 6.27 µg/ml) inhibited alpha-glucosidase. These results indicated that the crude, *E. undulata* acetone extract does contain compounds that display hypoglycaemic activity.

The hypoglycaemic activity of four plant species including *E. undulata*, and the four isolated purified compounds, are reported for the first time.

Table of Contents

DECLARATION	2
SUMMARY	3
CHAPTER 1.	
DIABETES:LITERATURE REVIEW	14
1.1. INTRODUCTION	14
1.2. CLASSIFICATION OF DIABETES MELLITUS	15
1.2.1 Insulin dependant diabetes mellitus (IDDM)	16
1.2.2 Non-insulin dependant diabetes mellitus (NIDDM)	17
1.3. DIABETES MELLITUS IN AFRICA	18
1.4. RATIONALE	19
1.5. PATHOPHYSIOLOGY OF DIABETES MELLITUS	19
1.5.1 Physiological mechanisms and management	19
1.5.1.1 The endocrine pancreas.....	19
1.5.1.2 Diabetes-related islet changes.....	20
1.5.2 Insulin	20
1.5.2.1 Insulin secretion	21
1.5.2.2 Insulin degradation	22
1.5.2.3 The insulin receptor.....	23
1.5.2.4 Effects of insulin on its targets.....	23
1.5.2.5 Action of insulin on glucose transporters (GLUT).....	23
1.5.2.6 Action of insulin on liver	24
1.5.2.7 Effect of insulin on muscle.....	25
1.5.2.8 Effect of insulin on adipose tissue.....	25
1.6 Complications of insulin therapy	26
1.7 Oral antidiabetic agents	26
1.7.1 Insulin secretagogues: sulfonylureas.....	26
1.7.2 Insulin secretagogues: meglitinides.....	26
1.7.3 Biguanides	28
1.7.4 Thiazolidinediones	28
1.7.5 Alpha-glucosidase inhibitors.....	30
1.8 Herbal products currently available in South Africa for the treatment of diabetes	30
1.9 Hypothesis and objectives of this study	31
1.10 Scope of this thesis	32
1.11 Conclusions	32
1.12 References	33

CHAPTER 2.

PLANT SPECIES USED IN THE TREATMENT OF DIABETES BY SOUTH AFRICAN TRADITIONAL HEALERS: AN INVENTORY38

2.1 Abstract	39
2.2 Introduction	39
2.3 Ethnobotany in South Africa	41
2.4 Plant Material	42
2.4.1. <i>Elaeodendron transvaalense</i>	42
2.4.1.1 Description.....	42
2.4.1.2 Medicinal uses.....	42
2.4.1.3 Phytochemistry / Bioactivity	43
2.4.2 <i>Euclea undulata</i>	43
2.4.2.1 Description.....	43
2.4.2.2 Medicinal uses.....	44
2.4.2.3 Phytochemistry / Bioactivity	44
2.4.3 <i>Euclea natalensis</i>	45
2.4.3.1 Description.....	45
2.4.3.2 Medicinal uses.....	45
2.4.3.3 Phytochemistry/ Bioactivity.....	46
2.4.4 <i>Lannea edulis</i>	46
2.4.4.1 Description.....	46
2.4.4.2 Medicinal uses.....	46
2.4.4.3 Phytochemistry / Bioactivity	47
2.4.5 <i>Spirostachys africanus</i>	47
2.4.5.1 Description.....	47
2.4.5.2 Medicinal uses.....	47
2.4.5.3 Phytochemistry / Bioactivity	47
2.4.6 <i>Schkuhria pinnata</i>	48
2.4.6.1 Description.....	48
2.4.6.2 Medicinal uses.....	48
2.4.7 <i>Pteronia divaricata</i>	49
2.4.7.1 Description.....	49
2.4.7.2 Medicinal uses	50
2.4.8 <i>Ziziphus mucronata</i>	50
2.4.8.1 Description.....	50
2.4.8.2 Medicinal uses.....	50
2.4.8.3 Phytochemistry / Bioactivity	51
2.4.9 <i>Aloe ferox</i>	51
2.4.9.1 Description.....	51

2.4.9.2 Medicinal uses.....	52
2.4.9.3 Phytochemistry / Bioactivity.....	52
2.4.10 <i>Warburgia salutaris</i>	52
2.4.10.1 Description	52
2.4.10.2 Medicinal uses	53
2.4.10.3 Phytochemistry / Bioactivity	53
2.4.11 <i>Momordica balsamina</i>	53
2.4.11.1 Description.....	54
2.4.11.2 Medicinal uses.....	54
2.4.11.3 Phytochemistry / Bioactivity	54
2.4.12 <i>Kederostis nana</i>	55
2.4.12.1 Description.....	55
2.4.12.2 Medicinal uses.....	55
2.4.13 <i>Artemisia afra</i>	56
2.4.13.1 Description.....	56
2.4.13.2 Medicinal uses.....	56
2.4.13.3 Phytochemistry / Bioactivity	57
2.4.14 <i>Catharanthus roseus</i>	57
2.4.14.1 Description	58
2.4.14.2 Medicinal uses	58
2.4.14.3 Phytochemistry / Bioactivity	58
2.4.15 <i>Cnicus benedictus</i>	58
2.4.15.1 Description	59
2.4.15.2 Medicinal uses	59
2.4.15.3 Phytochemistry / Bioactivity	59
2.4.16 <i>Psidium guajava</i>	60
2.4.16.1 Description.....	60
2.4.16.2. Medicinal uses	60
2.4.16.3 Phytochemistry / Bioactivity	60
2.4.17 <i>Terminalia sericea</i>	61
2.4.17.1 Description.....	61
2.4.17.2 Medicinal uses.....	61
2.4.17.3 Phytochemistry / Bioactivity	62
2.4.18 <i>Sutherlandia frutescens</i>	62
2.4.18.1 Description	62
2.4.18.2 Medicinal uses	62
2.4.18.3 Phytochemistry / Bioactivity	63
2.4.19 <i>Bridelia micrantha</i>	63
2.4.19.1 Description	63

2.4.19.2 Medicinal uses	64
2.4.19.3 Phytochemistry / Bioactivity	64
2.4.20 <i>Sclerocarya birrea</i>	64
2.4.20.1 Description	64
2.4.20.2 Medicinal uses.....	65
2.4.20.3 Phytochemistry / Bioactivity.....	65
2.4.21 <i>Brachylaena discolor</i>	65
2.4.21.1 Description	65
2.4.21.2 Medicinal uses	66
2.4.21.3 Phytochemistry / Bioactivity	66
2.4.22 <i>Brachylaena elliptica</i>	66
2.4.22.1 Description	66
2.4.22.2 Medicinal uses	67
2.4.22.3 Phytochemistry / Bioactivity	67
2.4.23 <i>Brachylaena ilicifolia</i>	67
2.4.23.1 Description	67
2.4.23.2 Medicinal uses	68
2.4.23.3 Phytochemistry / Bioactivity	68
2.4.24 <i>Bulbine latifolia</i>	68
2.4.24.1 Description.....	68
2.4.24.2 Medicinal uses.....	68
2.4.24.3 Phytochemistry / Bioactivity	69
2.4.25 <i>Carpobrotus edulis</i>	69
2.4.25.1 Description	69
2.4.25.2 Medicinal uses	69
2.4.25.3 Phytochemistry / Bioactivity	70
2.4.26 <i>Chironia baccifera</i>	70
2.4.26.1 Description	70
2.4.26.2 Medicinal uses	70
2.4.26.3 Phytochemistry / Bioactivity	71
2.4.27 <i>Cissampelos capensis</i>	71
2.4.27.1 Description	71
2.4.27.2 Medicinal uses.....	71
2.4.27.3 Phytochemistry / Bioactivity	72
2.4.28 <i>Harpagophytum procumbens</i>	72
2.4.28.1 Description	72
2.4.28.2 Medicinal uses	72
2.4.28.3 Phytochemistry / Bioactivity	73
2.4.29 <i>Hoodia currorii</i>	73

2.4.29.1 Description	73
2.4.29.2 Medicinal uses	73
2.4.29.3 Phytochemistry / Bioactivity	74
2.4.30 <i>Nymphaea nouchali</i>	74
2.4.30.1 Description	74
2.4.30.2 Medicinal uses	74
2.4.30.3 Phytochemistry / Bioactivity	75
2.4.31 <i>Trigonella foenumgraecum</i>	75
2.4.31.1 Description	75
2.4.31.2 Medicinal uses	75
2.4.31.3 Phytochemistry / Bioactivity	75
2.4.32 <i>Vernonia oligocephala</i>	76
2.4.32.1 Description	76
2.4.32.2 Medicinal uses	76
2.4.32.3 Phytochemistry / Bioactivity	76
2.5 Conclusion	77
2.6 Acknowledgement.....	78
2.7 References	78

CHAPTER 3.

HYPOGLYCAEMIC ACTIVITY OF FOUR PLANT EXTRACTS TRADITIONALLY USED IN SOUTH AFRICA FOR DIABETES	86
3.1 Abstract	87
3.2 Introduction	88
3.3 Material and Methods	89
3.3.1 Plant material	89
3.3.2 Preparation of plant extracts	90
3.3.3 <i>In vitro</i> anti-diabetic and toxicity screening.....	91
3.3.4 Routine maintenance of cell cultures	91
3.3.4.1 Glucose uptake experimental procedures on C2C12 myoblast cells	91
3.3.4.2 Glucose uptake experimental procedure on Chang..... liver cells and 3T3-L1 adipose cells	92
3.3.4.3 Dose response	93
3.4 Alpha-glucosidase inhibiting activity	93
3.5 Alpha-amylase inhibiting activity	94
3.5.1 Enzyme assay	94
3.5.2 Prussian blue assay	95
3.6 Results	95
3.6.1 <i>In vitro</i>	95
3.6.2 MTT toxicity assay	98
3.6.3 Alpha-glucosidase and alpha-amalyse assay	99

3.7 Discussion	100
3.8 Acknowledgements	102
3.9 References	102

CHAPTER 4.

ISOLATION OF BIOACTIVE COMPOUNDS FROM *EUCLEA UNDULATA* var. *MYRTINA* ROOTBARK.107

4.1 Abstract	107
4.2 Introduction	107
4.3 Material and methods	107
4.3.1 Plant material	107
4.3.2 Extraction of plant material	108
4.3.3 Fractionation of the crude plant extract	108
4.4 Determination of hypoglycaemic activity	108
4.5 Results and discussion	112
4.5.1 <i>In vitro</i> test results	112
4.5.2 Alpha glycosidase assay	112
4.6 Phytochemical examination	116
4.6.1 Chemical constituents from the root bark of <i>E. undulata</i>	116
4.6.1.1 α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	116
4.6.1.2 Betulin (2).....	123
4.6.1.3 Lupeol (3).....	123
4.6.1.4 Epicatechin (4)	126
4.7 Conclusions	128
4.8 References	129

CHAPTER 5.

GENERAL DISCUSSION AND CONCLUSION.....133

CHAPTER 6.

ACKNOWLEDGEMENTS AND PUBLICATIONS138

6.1 Congresses attended	138
6.2 Publications from this thesis	139

CHAPTER 7.

REFERENCES140

CHAPTER 8.

APPENDIX158

List of Figures

Chapter 1.

- Figure 1.1: Structure of human proinsulin. Insulin is shown as the shaded peptide chains, A and B (Davis and Granner, 1996).21
- Figure 1.2: Model of the control of insulin release from the pancreatic β -cells by glucose (Nolte and Karam, 2001).....22
- Figure 1.3: Schematic diagram of the probable structure of the insulin receptor tetramer in the activated state (Nolte and Karam, 2001).....24
- Figure 1.4: Chemical structure of the first generation sulfonylurea (a)tolbutamide, (b) tolazamide (c) chlorproamide and the second generation sulfonylurea (d) glyburide (e) glipzide and (f) glimepiride (PubChem Public Chemical Database, 2009).....26
- Figure 1.5: Chemical structure of the meglitinide repaglinide (PubChem Public Chemical Database, 2009).....27
- Figure 1.6: Chemical structure of the biguanide metformin (PubChem Public Chemical Database, 2009).....28
- Figure 1.7 *Galega officinalis* Van Wyk and Wink (2004).....28
- Figure 1.8: Chemical structure of the thiazolidinediones, a) troglitazone, b) rosiglitazone and c) pioglitazone (PubChem Public Chemical Database, 2009).....29

Chapter 3

- Figure 3.1. Glucose uptake (as % of control \pm standard error of mean, N = 8 for C2C12 and 10 for Chang liver and 3T3-L1) of the various plant extracts tested for their hypoglycaemic activity.....96
- Figure 3.2. Toxicity (as % of control \pm standard error of mean, N = 6) of the various plant extracts tested for their hypoglycaemic activity.....97
- Figure 3.3. Dose response of the *E. undulata* plant extract *in vitro* on Chang liver cells (as % of control \pm standard error of mean, N = 5). Cells were exposed to 12.5 μ g/ml extract for 48 hours before glucose utilization was measured in the presence of the extract concentrations as indicated. * $p < 0.05$; ** $p < 0.005$ compared to untreated control (using student's two-tailed t-test).....99

Chapter 4

Figure 4.1. Silica gel column fractionation of the acetone extract of <i>E. undulata</i>	109
Figure 4.2. Dendrogram indicating the isolation of compounds from the crude acetone extract of <i>E. undulata</i> var. <i>myrtina</i>	110
Figure 4.3. Glucose uptake (as % of control \pm standard error of mean N= 8) of the different fractions of the crude plant extract of <i>E. undulata</i> (12.5 μ g/ml) tested for their hypoglycaemic activity on C2C12 myocytes.....	114
Figure 4.4. Glucose uptake (as % of control \pm standard error of mean N=8) of the compounds isolated from <i>E. undulata</i> tested for their hypoglycaemic activity on C2C12 myocytes.....	113
Figure 4.5. Inhibition of alpha-glucosidase of the various compounds isolated from the acetone extract of <i>E. undulata</i> tested for (Bars represent the means of duplicate determinations and error bars the highest and lowest values).....	115
Figure 4.6 HMBC correlations of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	117
Figure 4.7 IR spectra of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	117
Figure 4.8. The ^1H -NMR spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	119
Figure 4.9. The ^{13}C NMR spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	120
Figure 4.10. - + ESI spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	121
Figure 4.11. The HMBC spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	122
Figure 4.12. The HSQC spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)	122
Figure 4.13. ^1H -NMR spectrum of betulin (2)	124
Figure 4.14. ^1H -NMR spectrum of lupeol (3).....	125
Figure 4.15 ^1H -NMR spectrum of epicatechin (4).....	127

List of Tables

Chapter 3.

Table 3.1. Effect of plant extracts on glucose utilization and toxicity on Chang liver cells, 3T3-L1 preadipocytes and C2C12 myocytes using the scoring system of Van De Venter *et al.* (2008)..... .98

Table 3.2. Inhibitory effect (IC_{50}) of four plant extracts and positive control acarbose for alpha-glucosidase and alpha-amylase.....100

Chapter 4.

Table 4.1 Inhibitory affect (IC_{50}) of the four compounds and positive control acarbose for alpha-glucosidase115

Table 4.2 NMR data of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1).....118

DIABETES : LITERATURE REVIEW

1.1 INTRODUCTION

Diabetes mellitus is recognized as being a syndrome, a collection of disorders that have hyperglycaemia and glucose intolerance as their hallmark, due either to insulin deficiency or to the impaired effectiveness of insulin's action, or to a combination of these. In order to understand diabetes it is necessary to understand the normal physiological process occurring during and after a meal. Food passes through the digestive system, where nutrients, including proteins, fat and carbohydrates are absorbed into the bloodstream. The presence of sugar, a carbohydrate, signals to the endocrine pancreas to secrete the hormone insulin. Insulin causes the uptake and storage of sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues (Roussel, 1998).

Unfortunately, there is no cure for diabetes yet but by controlling blood sugar levels through a healthy diet, exercise and medication the risk of long-term diabetes complications can be decreased. Long-term complications that can be experienced are:

- eyes – cataracts and retinopathy (gradual damaging of the eye) that may lead to blindness
- kidneys – kidney disease and kidney failure
- nerves – neuropathy (gradual damaging of nerves)
- feet – ulcers, infections, gangrene, etc.
- cardiovascular system – hardening of arteries, heart disease and stroke (Heart foundation, 2003).

The progressive nature of the disease necessitates constant reassessment of glycaemic control in people with diabetes and appropriate adjustment of therapeutic regimens. When glycaemic control is no longer maintained with a single agent, the addition of a second or third drug is usually more effective than switching to another single agent.

Medicinal plants which have showed anti-diabetic activity during earlier investigations include *Panax* species, *Phyllanthus* species, *Acacia arabica*, *Aloe vera*, *Aloe barbadensis*, *Artemisia pallens*, *Momordica charantia*, *Alium cepa*, *Trigonella foenum-graecum* etc (Soumyanath, 2006). Very few South-African plants have been scientifically analyzed for their anti-diabetic characteristics. The most recent work was done by Van Huyssteen (2007) and Van de Venter *et al.* (2008).

1.2. CLASSIFICATION OF DIABETES MELLITUS

A major requirement for orderly epidemiologic and clinical research on and for the management of diabetes mellitus is an appropriate classification. Furthermore the process of understanding the etiology of a disease and studying its natural history involves the ability to identify and differentiate between its various forms and place them into a rational etiopathologic framework (Harris and Zimmet, 1997).

The contemporary classification of diabetes and other categories of glucose intolerance, based on research on this heterogeneous syndrome, was developed in 1979 by the National Diabetes Data Group. Two major forms of diabetes are recognized in Western countries; insulin dependent diabetes mellitus (IDDM, type I diabetes) and non-insulin dependant diabetes (NIDDM, type II diabetes). The evidence of this heterogeneity is overwhelming and includes the following:

- a) there are many distinct disorders, most of which are individually rare, in which glucose intolerance is a feature;
- b) there are large differences in the prevalence of the major forms of diabetes among various racial or ethnic groups world-wide;
- c) glucose tolerance presents variable clinical features, for example, the differences between thin ketosis-prone, insulin dependant diabetes and obese, non-ketotic insulin resistant diabetes;
- d) genetic, immunologic and clinical studies show that in Western countries, the forms of diabetes with their onset primarily in youth or in adulthood are distinct entities;

- e) the type of non-insulin requiring diabetes in young people, which is inherited in an autosomal dominant fashion is clearly different from the classic acute diabetes of juveniles; and
- f) in tropical countries, several clinical presentations occur, including fibrocalcific pancreatitis and malnutrition-related diabetes.

This and other collective evidence have been used to divide diabetes mellitus into four distinct types namely;

- insulin dependant diabetes,
- non-insulin dependant diabetes,
- malnutrition-related diabetes,
- other types of diabetes.

The classification highlights the marked heterogeneity of the diabetic syndrome. Such heterogeneity has important implications not only for clinical management of diabetes but also for biomedical research (Harris and Zimmet, 1997). In this study the focus was mainly on type II diabetes while type I diabetes was discussed briefly to point out the differences between the two types of diabetes.

1.2.1 Insulin dependant diabetes mellitus (IDDM)

The subclass of diabetes, type I diabetes, is generally characterized by the abrupt onset of severe symptoms, dependence on exogenous insulin to sustain life and proneness to ketosis even in the basal state, all of which is caused by absolute insulin deficiency. IDDM is the most prevalent type of diabetes among children and young adults in developing countries, and was formally termed juvenile diabetes (Harris and Zimmet, 1997). It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated, and the pancreatic B cells fail to respond to all insulinogenic stimuli (Nolte and Karam, 2001).

Type I diabetes is thought to result from an infectious or toxic environmental contingency in people whose immune systems are genetically predisposed to develop a vigorous autoimmune response against pancreatic B cell antigens. Extrinsic factors that might affect

B cell functioning include damage caused by viruses such as the mumps virus and coxsackie virus B4, by chemical agents, or by destructive cytotoxins and antibodies released from sensitized immunocytes. An underlying genetic defect relating to pancreatic B cell replication or function may predispose a person to the development of B cell failure after viral infections. In addition, specific HLA genes may increase susceptibility to a diabetogenic virus or may be linked to certain immune response genes that predispose patients to a destructive autoimmune response against their own islet cells (auto-aggression). Observations that pancreatic B cell damage appears to be lessened when immunosuppressive drugs such as cyclosporine or azathioprine are given at the initial manifestation of type I diabetes support the importance of auto-aggression by the immune system as a major factor in the pathogenesis of this type of diabetes (Nolte and Karam, 2001).

1.2.2 Non-insulin dependant diabetes mellitus (NIDDM)

Type II diabetes greatly out numbers all other forms of diabetes. Patients with NIDDM are not dependant on exogenous insulin for prevention of ketonuria and are not prone to ketosis. However, they may require insulin for the correction of fasting hyperglycaemia if this cannot be achieved with the use of diet or oral agents, and they may develop ketosis under special circumstances such as severe stress precipitated by infections or trauma (Harris and Zimmet, 1997).

The pathogenesis in type II diabetes is that the pancreas produces insulin but the body does not utilize the insulin correctly. This is primarily due to peripheral tissue insulin resistance where insulin-receptors or other intermediates in the insulin signaling pathways within body cells are insensitive to insulin and consequently glucose does not readily enter the tissue leading to hyperglycaemia or elevated blood glucose concentrations (Albright, 1997). Obesity, which generally results in impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type II diabetes are obese (Nolte and Karan, 2001) and will ultimately require multiple anti-diabetic agents to maintain adequate glycaemic control (Gerich, 2001).

1.3 DIABETES MELLITUS IN AFRICA

Most countries in Africa are undergoing a demographic transition, and African urban societies are increasingly coming within the sphere of the influence of Western market economies. The lifestyle of city dwellers tends to be material-behavioural, with the adoption of cosmopolitan behaviour and consumption of resources and food, especially fast foods. This has led to an increase in the consumption of fat, sugar and salt. Rural African societies, however, have seen an increase in nutritional deficiencies, which appear to be related to drought, poverty, war and socio-economic deprivation rather than to culture or religion. In these rural areas, the focus has been on maintaining food availability rather than equitable distribution. Lifestyle changes and a very rapid increase in the urban population of Africa has led to inadequate production of local cereals and staples like sorghum, millet, maize, yam and plantain. This phenomenon has led to many countries having a low daily *per capita* dietary energy supply, and has led to a difference in food patterns between the urban and rural dwellers and the occurrence of diabetes mellitus. These lifestyle changes have evolved against a background of increasing prevalence of diabetes mellitus and diabetic complications in Africa (Yajnik, 1990; King and Rewers, 1993).

In South Africa a number of studies have been conducted and it is estimated that there are at least 6.5 million known diabetics and possibly up to an equal number who are currently undiagnosed (Healt 24, 2006). The prevalence of diabetes in South Africa is high, and is estimated to be 14% in the Coloured community, 13% in the Indian community, 6% in the African community and 6% in the European community (Society of Endocrinology, Metabolism and Diabetes in South Africa, 2003).

There is certainly, a demand for more nutrition education of the more cosmopolitan diabetic population by the limited number of poorly equipped staff who need to formulate new approaches that are more relevant to the needs of their patients (Mbanya and Gwangwa'a, 1997).

1.4 RATIONALE

The first description of diabetes is credited to Arataeus of Cappadocia in Asia Minor in the first century AD. The first attempts for treating diabetes, when no more was known about it than the polyuria, were made by Dr. John Rollo, Surgeon General to the Royal Artillery, 1796 through dietary restrictions. The discovery of insulin in 1921 by Dr Frederick Banting was a major breakthrough in the history of medicine and the treatment of diabetes (Pyke, 1997).

Diabetes mellitus (DM) is the most common endocrine disorder, and affects more than 100 million people worldwide (6% of the population) and in the next 10 years it may affect five times more people than it does now (World Health Organization and American Diabetes Association). The World Health Organization has pointed out that the prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be an attainable target, and strongly emphasize the optimal, rational use of traditional and natural indigenous medicines (World Health Organization 1985, 1994).

Though the development of modern medicine resulted in the advent of modern pharmaceuticals including insulin, biguanides, sulfonylureas and thiazolidinediones there is still a need to look for new drugs as no drug (except strict glycaemic management with insulin) has been shown to control diabetic complications effectively. In relation to plants also, barring a few studies, most of the studies have not assessed the impact of these plants on the course of diabetic complications. It is necessary to evaluate plant species traditionally used against diabetes mellitus to discover new compounds that can be used effectively against this disease as well as lessen diabetic complications.

1.5. PATHOPHYSIOLOGY OF DIABETES MELLITUS

1.5.1 Physiological mechanisms and management

1.5.1.1 The Endocrine pancreas

The human pancreas is basically composed of two types of secretory cells that are both involved in nutrient handling: 98% of the cells- the exocrine type – secrete a food-processing enzyme-bicarbonate mixture into the duodenum, while the remaining 2% - the endocrine type- have a metabolic function and secrete a mixture of nutrient-generated hormones into the portal vein. This small endocrine part is of vital importance in maintaining glucose homeostasis through the action of the 51-amino acid peptide insulin. Four endocrine cell types can be distinguished: A cells (alpha), B cells (beta), D cells (delta) and PP cells (pancreatic polypeptide) (Klöppel and In't Veld, 1997). These endocrine cells are distributed throughout the pancreas in areas known as islets.

1.5.1.2 Diabetes-related islet changes

The islet changes, from a morphological point of view, associated with various types of diabetes can be divided into those with and without severe beta-cell loss. Severe beta-cell loss is found in type I diabetes and some uncommon forms of diabetes such as virus-related diabetes and congenital diabetes. Islets without severe loss of beta-cells are encountered in type II diabetes and in the secondary forms of diabetes (Klöppel and In't Veld, 1997).

1.5.2 Insulin

The beta-cells of the pancreatic islets synthesize insulin from a single chain precursor of 110 amino acids termed preproinsulin. After translocation through the membrane of the rough endoplasmic reticulum, the 24-amino-acid N-terminal signal peptide of preproinsulin is rapidly cleaved off to form proinsulin. Here the molecule folds and the disulfide bonds are formed. On the conversion of human proinsulin to insulin in the Golgi-complex, four basic amino acids and the remaining connector or C peptide are removed by proteolysis. This gives rise to the two-peptide chains (A and B) of the insulin molecule, which contains one intra-subunit and two inter-subunit disulfide bonds. The A chain usually is composed of 21 amino acids and the B chain 30. The two chains of insulin form a highly ordered structure with several α helical regions in both the A and B chains (Figure 1.1).

Two ions of Zn^{2+} are coordinated in a proinsulin hexamer and this form of insulin presumably is stored in the granules of the pancreatic β cells. It is believed that Zn^{2+} has a

1.5.2.2 Insulin degradation

The liver and kidney are the two main organs that remove insulin from circulation, presumably by hydrolysis of the disulfide connection between the A and B chains through the action of glutathione insulin transhydrogenase (insulinase). After this reductive cleavage further degradation by proteolysis occurs. The liver normally clears the blood of

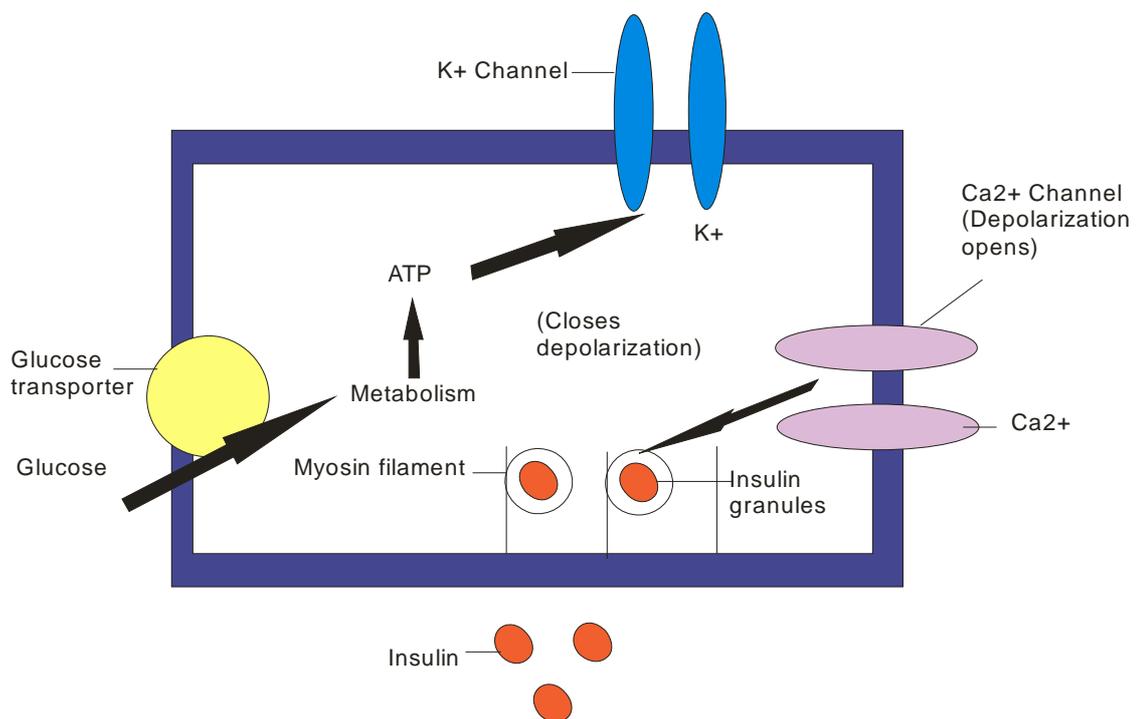


Figure 1.2. Model of the control of insulin release from the pancreatic β -cells by glucose (Nolte and Karam, 2001).

approximately 60% of the insulin released from the pancreas by virtue of its location as the terminal site of the portal vein blood flow, with the kidneys removing 35 – 40% of the endogenous hormone. However, in insulin-treated diabetics receiving subcutaneous insulin injections, this ratio is reversed, with 60% of exogenous insulin being cleared by the kidney and the liver removing no more than 30-40%. The half-life of circulating insulin is 3-5 minutes (Nolte and Karam, 2001).

1.5.2.3 The insulin receptor

Once insulin has entered the circulation, it is bound by specialized receptors that are found on the membranes of most cells. However, the biological responses promoted by these insulin–receptor complexes have only been identified in a few target tissues, e.g. liver, muscle and adipose tissue. The receptors bind insulin with high specificity and affinity in the picomolar range. The full insulin receptor consists of two heterodimers, each containing an alpha subunit, which is entirely extra cellular and constitutes the recognition site, and a beta subunit that spans the membrane (Figure 1.3). The β - subunit contains a tyrosine kinase. When insulin binds to the alpha subunit on the outer surface of the cell, tyrosine kinase activity is stimulated in the beta portion. Although the $\alpha\beta$ dimeric form is capable of binding insulin, it does so with a much lower affinity than the tetrameric $\alpha\alpha\beta\beta$ form. Self-phosphorylation of the β portion of the receptor causes both increased aggregation of $\alpha\beta$ heterodimers and stabilization of the activated state of the receptor tyrosine kinase.

In clinical situations associated with elevated levels of circulating insulin, such as obesity or insulinoma, the concentration of insulin receptors is reduced. This down regulation of insulin receptors seems to provide an intrinsic mechanism whereby the target cells limit their response to excessive hormone concentrations (Nolte and Karam, 2001).

1.5.2.4 Effects of insulin on its targets

Insulin promotes the storage of fat as well as glucose within specialized target cells and influences cell growth and the metabolic functions of a wide variety of tissues.

1.5.2.5 Action of insulin on glucose transporters (GLUT)

Insulin has an important effect on several transport molecules that facilitate glucose movement across cell membranes. These transporters may play a role in the etiology as well as the manifestation of diabetes. GLUT 4, quantitatively the most important in terms of lowering blood glucose, is inserted into the membranes of muscle and adipose cells from intracellular storage vesicles by insulin. Defects in GLUT 2 mediated transport of glucose into pancreatic β -cells may contribute to the reduced insulin secretion that characterizes type II diabetes.

1.5.2.6 Action of insulin on the liver

The first major organ reached by endogenous insulin via the portal circulation is the liver, where its function is to increase storage of glucose as glycogen and to reset the liver to the fed state by reversing a number of catabolic mechanisms, such as glycogenolysis,

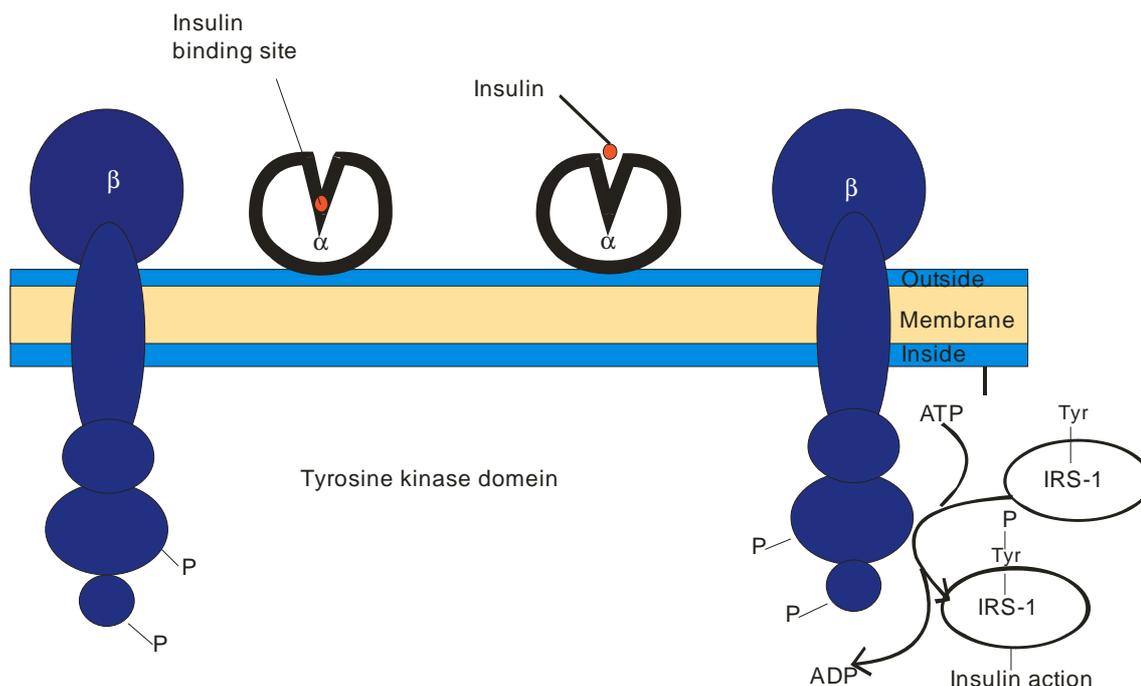


Figure 1.3. Schematic diagram of the probable structure of the insulin receptor tetramer in the activated state (Nolte and Karam, 2001).

ketogenesis, and gluconeogenesis, which are associated with the post-absorptive state. These effects are brought about directly through insulin-induced phosphorylations, which activate pyruvate kinase, phosphofruktokinase and glucokinase, while relieving gluconeogenic enzymes, including pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose bisphosphatase, and glucose 6-phosphatase. Insulin also exerts indirect effects to decrease hepatic gluconeogenesis and ketogenesis by reducing the fatty acid flux to the liver through its antilipolytic action on adipocytes. In addition, insulin decreases urea production, protein catabolism, cAMP (cyclic adenosine monophosphate) in the liver, promotes triglyceride synthesis and, increases potassium and phosphate uptake by the liver.

1.5.2.7 Effect of insulin on muscle

Insulin promotes protein synthesis by increasing the amino acid transport and by stimulating ribosomal activity. It also promotes glycogen synthesis to replace the glycogen stores expended by muscle activity. This is accomplished by increasing the glucose transport into the muscle cells, inducing glycogen synthase, and inhibiting glycogen phosphorylase.

1.5.2.8 Effect of insulin on adipose tissue

Insulin acts on reducing circulating free fatty acids and promoting triglyceride storage in adipocytes by three mechanisms:

- 1) induction of lipoprotein lipase, which actively hydrolyzes triglycerides from circulating lipoproteins;
- 2) glucose transport into cells to generate glycerophosphate as a metabolic product, which permits esterification of fatty acids supplied by lipoprotein hydrolysis; and
- 3) reduction of intracellular lipolysis of stored triglyceride by a direct inhibition of intracellular lipase (Nolte and Karam, 2001).

1.6 COMPLICATIONS OF INSULIN THERAPY

Oral hypoglycaemic agents/insulin is the mainstay of the treatment of diabetes and is effective in controlling hyperglycaemia. However, it has prominent side effects and fails to significantly alter the course of diabetic complications (Rang and Dale, 1991). The main complications of insulin therapy are 1) Hypoglycaemia which may result from a delay in taking a meal, unusual physical exertion or a dose of insulin that is too large for immediate needs. Autonomic warning signals of hypoglycaemia and the manifestation of insulin excess are mainly those of impaired functions of the central nervous system such as mental confusion, bizarre behaviour and ultimately coma. More rapid development of hypoglycaemia from the effects of regular insulin use causes signs of autonomic hyperactivity, both sympathetic (tachycardia, palpitations, sweating, tremulousness) and parasympathetic (nausea, hunger) that may progress to convulsions and coma if untreated.

2) Immunopathology of insulin therapy includes i) insulin allergy, ii) immune insulin

resistance (development of anti-insulin antibodies) and iii) lipodystrophy at injection sites (Nolte and Karam, 2001).

1.7 ORAL ANTIDIABETIC AGENTS

Four categories of oral antidiabetic agents are available namely; insulin secretagogues, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors (Nolte and Karam, 2001).

1.7.1 Insulin secretagogues: sulfonylureas

The major action of sulfonylureas is to increase insulin release from the pancreas. Sulfonylureas binds to a 140kDa high-affinity sulfonylurea receptor that is associated with a beta cell inward rectifier-type ATP-sensitive potassium channel. The binding of a sulfonylurea inhibits the efflux of potassium ions through the channel and results in depolarization. Depolarization, in turn, opens a voltage-gated calcium channel that results in a calcium influx and the release of insulin. Insulin synthesis is not stimulated and may even be reduced by sulfonylureas. Some evidence indicates that after prolonged sulfonylurea therapy, serum insulin levels no longer increase but may even decrease. It was also established that chronic administration of sulfonylureas to type 2 diabetic patients reduced serum glucagon levels but increased the binding of insulin to the tissue receptors (Nolte and Karam, 2001). Seven sulfonylurea drugs are available in the USA and are conventionally divided into first and second generation agents, which differ primarily in their potency. The first-generation includes tolbutamide, tolazamide, acetohexamide and chlorpropamide and the second generation includes glyburide, glipizide and glimepiride.

1.7.2 Insulin secretagogues: meglitinides

Meglitinides are a new class of insulin secretagogues. Repaglinide, the first member of the group, was approved for clinical use by the FDA in 1998. These drugs modulate Beta cell insulin release by regulating potassium efflux through the potassium channels. Meglitinides and sulfonylureas overlap in their molecular binding sites since meglitinides have two binding sites in common with sulfonylureas and one unique binding site. They have however, no direct effect on insulin exocytosis as does sulfonylureas (Nolte and Karam, 2001).

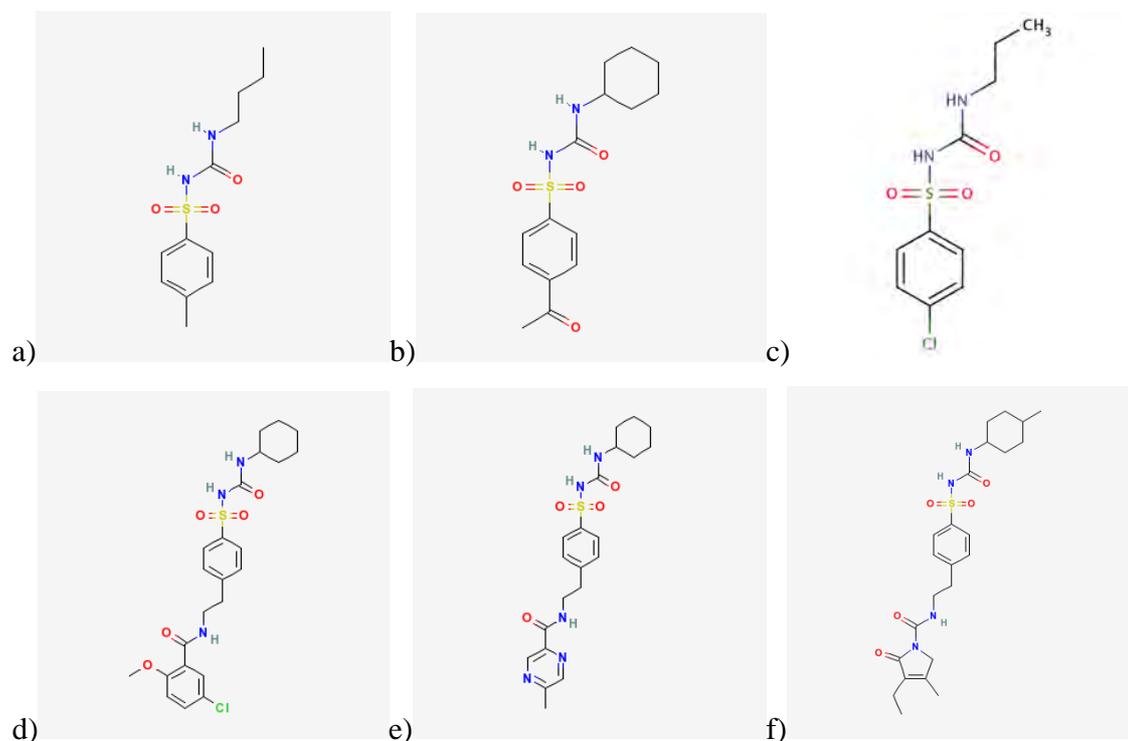


Figure 1.4 Chemical structure of the first generation sulfonylurea (a) tolbutamide, (b) tolazamide (c) chlorpropamide and the second generation sulfonylurea (d) glyburide (e) glipizide and (f) glimepiride (PubChem Public Chemical Database, 2009).

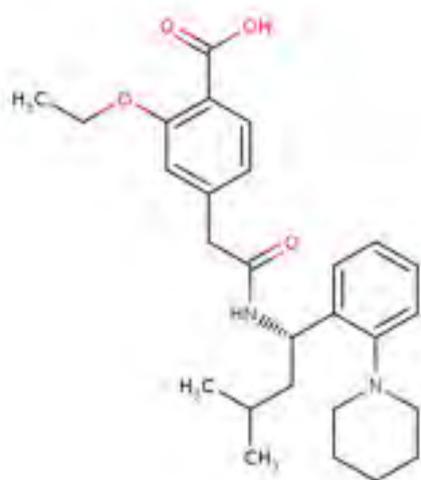


Fig 1.5 Chemical structure of the meglitinide repaglinide (PubChem Public Chemical Database, 2009).

1.7.3 Biguanides

Three types of biguanides are being used in the treatment of diabetes namely phenformin, buformin and metformin. The use of the first two mentioned was discontinued in the United States of America due to its association with lactic acidosis (Nolte and Karam, 2001).

Metformin originates from the French lilac, *Galega officinalis* L., a perennial herb known for centuries to reduce the symptoms of diabetes. The active compound is galegine, a guanidine derivative. Metformin's clinical trials were successfully completed in 1995 and its use approved in the United States of America. The full extent of the mechanism of the action of biguanides is unknown, but its blood glucose-lowering action does not depend on the presence of functioning pancreatic beta cells. Proposed mechanisms of action includes direct stimulation of glycolysis in the tissue, and the increase of glucose removal from the blood; reduced hepatic gluconeogenesis; slowing of glucose absorption from the gastrointestinal tract; with increase glucose to lactate conversion by enterocytes and the reduction of plasmaglucon levels (Nolte and Karam, 2001).

Biguanides have been most often prescribed for patients with refractory obesity whose hyperglycemia is due to insulin resistance. As metformin is an insulin-sparing agent and does not increase weight or provoke hypoglycemia it has the advantage over insulin and sulfonylureas in treating hyperglycemia. The most frequent toxic affects of metformin are gastrointestinal and there is a risk of lactic acidosis.

1.7.4 Thiazolidinediones

Thiazolidinediones is a recently introduced class of oral antidiabetic drug that enhances target tissue insulin sensitivity. Two types are commercially available namely rosiglitazone and pioglitazone. The exact mechanism of their action is not known, but their major action is to diminish insulin resistance in muscle and adipose tissue.

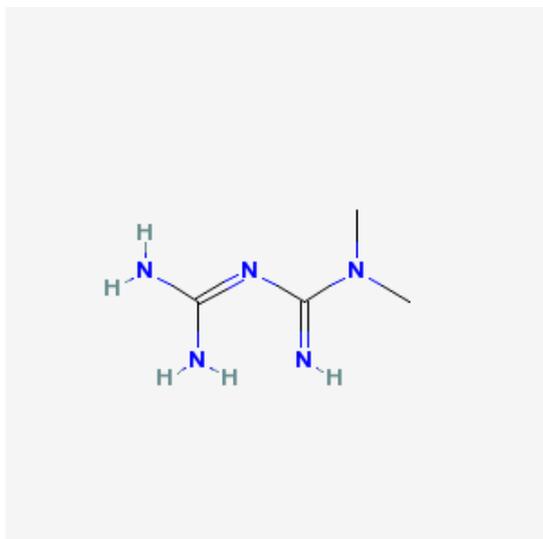


Fig 1.6 Chemical structure of the biguanide metformin (PubChem Public Chemical Database, 2009).



Figure 1.7 *Galega officinalis* Van Wyk and Wink (2004).

Troglitazone was the first thiazolidinedione to be approved but was withdrawn due to its association with a low but significant rate of idiosyncratic liver damage. Two other thiazolidinediones namely rosiglitazone and pioglitazone demonstrated efficacy similar to that of troglitazone but with no evidence of hepatotoxicity (Nolte and Karam, 2001).

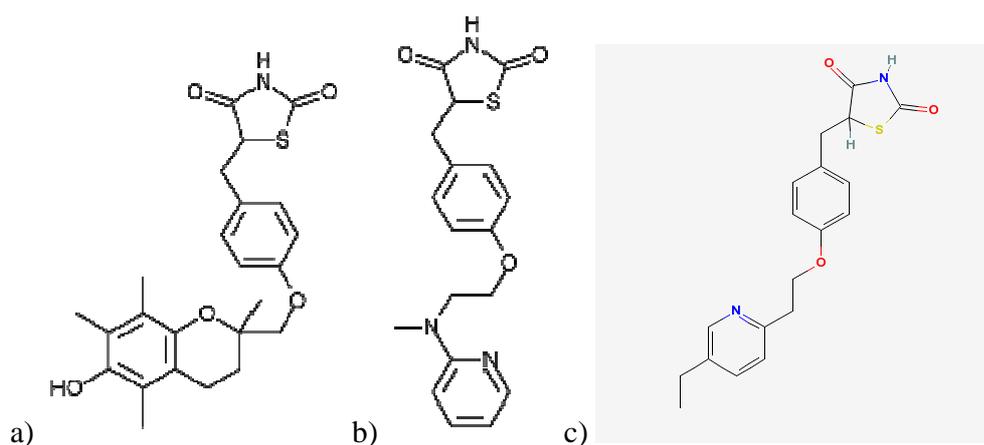


Fig 1.8 Chemical structure of the thiazolidinediones, a) troglitazone, b) rosiglitazone and c) pioglitazone (PubChem Public Chemical Database, 2009)

1.7.5 Alpha-glucosidase inhibitors

Acarbose and miglitol are the two agents available in this class. Alpha-glucosidase inhibitors act by inhibiting the enzymes, pancreatic alpha-amylase and alpha-glucosidase, found in the brush border cells that line the small intestine. They cleave the more complex carbohydrates such as starches, oligosaccharides and disaccharides into monosaccharide molecules before being absorbed in the duodenum and upper jejunum (Luna and Feinglos, 2001; Nolte and Karam, 2001). Acarbose and miglitol are competitive inhibitors of alpha-glucosidase and modulate the postprandial digestion and absorption of starch and disaccharides. Miglitol differs structurally from acarbose and is six time more potent in inhibiting sucrase. The binding affinity of the two compounds differ, acarbose and miglitol both target alpha-glucosidases: sucrase, maltase, glycoamylase, dextranase. Isomaltase and Beta-glucosidase are targeted only by miglitol and alpha-amylase only by acarbose. The clinical consequence of enzyme inhibition is to minimize upper intestinal digestion and absorption of ingested starch and disaccharides in the distal small intestine, lowering postmeal glycemic excursions and creating an insulin-sparing effect (Nolte and Karam, 2001). Prominent adverse effects include flatulence, diarrhoea, and abdominal pain which results from the appearance of undigested carbohydrate in the colon that is then fermented into short-chain fatty acids, releasing gas (Nolte and Karam, 2001).

1.8 HERBAL PRODUCTS CURRENTLY AVAILABLE IN SOUTH AFRICA FOR THE TREATMENT OF DIABETES.

Few herbal products are available on the South African market for the maintenance of blood glucose levels. The most commonly used includes Probetix, Manna, Diabecinn and Cinnachrome. Probetix (Nappi code: 711050-001) is a herbal supplement developed from the leaf extract of the indigenous shrub *Sutherlandia frutescens* (L.) R. Br.. Research done on this plant extract found that it reversed insulin resistance and decreased intestinal glucose uptake (Chadwick *et al.*, 2007). Scientifically identified biological active chemicals isolated from the seeds of *Sutherlandia frutescens* include L-canavanine, a non-protein α -amino acid and pinitol, the latter associated with hypoglycaemic effects (Van Wyk *et al.*, 2005).

Manna DFM43 (Nappi code 705846-001) is a high fibre, low fat nutritional food supplement developed from the pods of the invasive tree *Prosopis glandulosa* Torr. var. *torreyana* (L.D. Benson) M.C. Johnst. Research indicated that it retards the absorption of glucose in the blood and reduces the Glycaemic Index (GI) value of foods. The active ingredient is galactomannan, a polysaccharide combination of galactose and mannose (Dune Foods, 2005).

Diabecinn (Nappi code 704686-001) is a food supplement that may reduce blood sugar levels, triglycerides, LDL cholesterol and total cholesterol in patients with type 2 diabetes. Diabecinn is a waterbased cinnamon bark extract (ZN112) that has shown in animals to increase the *in vitro* glucose uptake and glycogen synthesis and to increase the phosphorylation of the insulin receptor. This increases insulin sensitivity, which improves blood glucose levels. Khan *et al.* 2003 stated that the dietary components beneficial in the prevention and treatment of type 2 diabetes and cardiovascular diseases have not been clearly defined, but it is postulated that spices may play a role. Spices such as cinnamon, cloves, bay leaves and turmeric display insulin-enhancing activity *in vitro*.

Cinnachrome (Nappi code 708102-001) contains the active ingredient cinnulin PF produced from cinnamon bark as well as the active substance methyl-hydroxy-chalcone polymer. It is alledged that Cinnachrome may assist in regulating blood sugar levels (Holford, 2009).

A number of South African plant species are being used traditionally for the treatment of diabetes, however, not many scientific studies have been conducted on these plant species. This necessitates researchers to investigate the potential of South African plant species for hypoglycaemic activity.

1.9 HYPOTHESIS AND OBJECTIVES OF THIS STUDY

The hypothesis of this thesis was that the different plant species used by traditional healers and herbalists for the treatment of diabetes would show hypoglycaemic activity.

The primary objectives of this study were to validate four plant species for their hypoglycaemic activity by

- a) evaluating their inhibiting effects on the carbohydrate-hydrolising enzymes, alpha-glucosidase and alpha-amylase,
- b) screening these plant extracts against C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells by measuring glucose uptake,
- c) the cytotoxicity of these plant extracts on cell lines
- d) the four plant species extracts according to a scoring system to establish which extracts are the best with regard to their hypoglycaemic potential.
- e) bioassay-guided fractionation of the active extract in order to identify the bioactive principles
- f) the isolated compounds for hypoglycaemic activity

1.10 SCOPE OF THIS THESIS

The scope of this thesis is a literature review on diabetes in Chapter 1. Chapter 2 deals with the importance of medicinal plants in general, and South Africa in particular. It also includes a literature review on plant species used in the treatment of diabetes. In chapter 3 the hypoglycaemic activity of the four selected plant species traditionally used in South Africa for the treatment of diabetes is discussed. Chapter 4 deals with the isolation of the bioactive compounds from *E. undulata* rootbark as well as the evaluation of these isolated compounds for hypoglycaemic activity. Chapter 5 comprises a general discussion and conclusion.

1.11 CONCLUSION

As the knowledge of the heterogeneity of diabetes mellitus increases, there is a need to look for more efficacious agents with fewer side effects. Complications are the major cause of morbidity and mortality in diabetes mellitus (Grover, Yadav and Vats, 2002). These complications include the specific diabetic problems of retinopathy, nephropathy and neuropathy which are often termed diabetic micro-angiopathic or microvascular disease and the non-specific macrovascular problems of occlusive atherosclerotic disease affecting the heart, brain and legs (Paul, 2002).

South Africa is a country with a rich diverse flora with a long history of use of indigenous plants by herbalists and traditional healers for the treatment of various diseases such as diabetes. According to Mulholand and Drewes (2004) 80% of the South African population is still making use of traditional medicines, coupled with a sympathetic attitude towards traditional healers. It is therefore, necessary that research should be done on the various plant species being used by traditional healers for treatment not only of diabetes but for other diseases as well, and to verify their activity and toxicity to possibly discover new effective drugs.

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An extensive literature review was conducted on the use of plants for the treatment of diabetes by South African herbalists and traditional healers. A brief description is given of each plant in Chapter 2. Appendix 1. contains photos of the plants described in Chapter 2.

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CHAPTER 2

Plant Species Used in the Treatment of Diabetes by South African Traditional Healers: An Inventory

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2.1 Abstract

The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes, with about 4,000 taxa being so employed. Traditional medicines continue to play a significant role in the treatment of life-threatening diseases such as malaria, tuberculosis, diabetes and AIDS in the developing world, although no adequate scientific evidence has been documented in support of their healing properties. The primary goal of this paper was to summarize information on some of the plants species used by traditional healers for the treatment of diabetes in South Africa. The information obtained is from published literature as well as personal communication with various traditional healers and herbalists from different areas. In total, the information of 32 plant species, representing 20 families, traditionally used by healers in the treatment of diabetes, is discussed, of which 14 are currently being investigated for their hypoglycemic activity by various scientists at the University of Pretoria.

Keywords: Diabetes, herbalists, plants, South Africa, traditional healers.

2.2 Introduction

Most African countries are undergoing a demographic transition and are increasingly coming under Western influences, leading to the adoption of a cosmopolitan lifestyle and a Western food culture, thus giving rise to an increase in the consumption of fat, sugar and salt. Rural African societies have maintained their traditional diet. However, an increase in nutritional deficiencies has occurred which appears to be related to drought, poverty, war and socioeconomic deprivation rather than to culture or religion. These lifestyle changes and a rapid increase in the urban population of Africa have led to an increase in nutritional deficiencies and, subsequently, a rise in the occurrence of diabetes mellitus (Mbanya and Gwangwa, 1997).

Diabetes mellitus is a common endocrine disorder, and affects more than 100 million people worldwide (World Health Organization, 1994). It is recognized as being a syndrome, a collection of disorders that have hyperglycaemia and glucose intolerance as a

hallmark, due either to insulin deficiency or to impaired effectiveness of insulin's action, or a combination of both.

Ethnopharmacological studies can contribute greatly to modern medicine, and can lead to the discovery of many novel and useful drugs, although the modern and the traditional uses may be entirely different (Holmstedt and Bruhn, 1995). The identification of biologically active compounds needs to be interpreted in the light of the traditional use and preparation of the plant (Holmstedt and Bruhn, 1995). It should comprise a chemical and pharmacological evaluation of the traditional drug preparation in order to establish dose-effect relationships for the quantitative use of the remedy.

The use of plants as medicine goes back to early man. Certainly, the great civilizations of the ancient Chinese, Indians, and Egyptians provided written evidence of man's ingenuity in utilizing plants for the treatment of a wide variety of diseases.

It was not until the 19th century that man began to isolate the active compounds of medicinal plants, and one particular landmark was the discovery of quinine from *Cinchona* bark by the French scientists Caventou and Pelletier. Such discoveries led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles in the quest for new medicines (Phillipson, 2001).

Prior to World War II, a series of natural products isolated from higher plants became clinical agents and a number are still in use today, such as morphine, codeine, digoxin, atropine and hyoscine. The antibiotic era dawned during and after World War II, with the discovery of the antibacterial effects of a whole series of natural products isolated from species of *Penicillium*, *Cephalosporium*, and *Streptomyces*. In the post-war years there were relatively few discoveries of new drugs from higher plants. Despite the discoveries of reserpine and vincristine, the impact of phytochemistry on new drug development waned and inevitably the innovative pharmaceutical industry turned to synthetic chemicals (Phillipson, 2001). During recent years, the attention of the pharmaceutical industry has switched once more to the natural world and this is illustrated by the development of three clinical drugs: taxol, etoposide, and artemisinin (Phillipson, 1999).

2.3 Ethnobotany in South Africa

The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes, with about 4,000 taxa being so employed. The trade in medicinal plants is an important part of the regional economy with over 700 plant species being reported as traded (Mander, 1998). In South Africa, it is estimated that there are 27 million consumers of traditional medicine (Mulholland and Drewes, 2004).

The value of trade in ethnomedicinal plants in KwaZulu-Natal alone was estimated to be worth R60 million in 1998. Most households spend between 4% and 8% of their annual income on traditional medicinal services. In addition, in KwaZulu-Natal, between 20,000 and 30,000 people derive an income from trading indigenous plants (Mulholland and Drewes, 2004).

The use of plants in traditional medicine finds its natural expression and further development in primary health care. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicine has often maintained popularity for historical and cultural reasons. Traditional medicines also continue to play a significant role in the treatment of life-threatening diseases such as malaria, tuberculosis and AIDS in the developing world, although no adequate scientific evidence has been documented in support of their healing properties.

The primary goal of this paper was to summarize information on some of the plants used by traditional healers and herbalists for the treatment of diabetes in South Africa. The information obtained is from published literature as well as personal communication with various traditional healers from different areas. The traditional healers and herbalists interviewed were recommended by healthcare professionals and local community workers active in the respective areas. Plant material used in this study was authenticated by Ms. M. Nel and Prof. A.E. van Wyk at the H.G.W.J. Schweickert Herbarium, University of Pretoria, where voucher specimens are being kept. In total, the information of 32 plant species representing 20 families traditionally used by healers in the treatment of diabetes is

discussed. The information on the first ten plants mentioned was provided by various traditional healers and herbalists. The medicinal use of the other 22 plants was obtained from literature.

2.4 Plant material

2.4.1 *Elaeodendron transvaalense*

Elaeodendron transvaalense (Burt Davy) R.H. Archer (Celastraceae); common names: Transvaal saffronwood (English); Transvaalsafraan (Afrikaans); ingwavuma (Zulu); mukuvhazwivhi (Venda). Plant parts used: bark.

2.4.1.1 Description

This is a shrub or small multi-stemmed tree, usually around 5 m tall but may reach 10 m or more. The bark is smooth and has a characteristic pale, grey colour. Tufts of leaves are crowded on the ends of rigid shoots. The leaves are oblong in shape with a firm texture and conspicuous venation on the upper and lower surfaces. The leaf margin is sometimes toothed. Small and inconspicuous greenish flowers are produced in summer, followed by oblong, yellow to dark orange, edible berry-like fruits (Coates Palgrave, 1984). The species is widely distributed in the northeastern parts of South Africa. It occurs along the coastal parts of KwaZulu-Natal and in Mpumalanga, Gauteng, and Limpopo.

2.4.1.2 Medicinal uses

An infusion of the bark is taken as a stomach cleanser and used as an enema for stomach aches, fever and to treat intestinal cramps and diarrhoea. The leaves are chewed and the juices swallowed for a sore throat (Van Wyk *et al.*, 2005). A teaspoon of powdered bark is boiled in water and no more than two cups are taken per day (Pujol, 1990). The powdered bark may also be licked from the palm of the hand, and washed down with a small amount of water. The bark is known to be toxic so the dosage should be carefully controlled. The species is used for the treatment of diabetes by local herbalists and

traditional healers in the Venda region, Limpopo (Dr. Emmanuel Tshikalange, Department of Plant Science, University of Pretoria, Pretoria, 0002, South Africa, personal communication).

2.4.1.3 Phytochemistry/bioactivity

The beneficial effects of the bark have been ascribed to its high tannin content (Frost, 1941). A phenolic compound, elaeocyanidin, has been isolated from both *E. transvaalense* and *E. croceum* (Thunb.) DC. (Drewes and Mashimbye, 1993). The latter species also contains gallotannins and ouratea proanthocyanidin A, and it is likely that these or similar compounds will be present in *E. transvaalense*, together with the reported triterpenoids (Drewes and Mashimbye, 1993). Tannins are sometimes used for their astringent and antidiarrhoeal properties (Bruneton, 1995). The bioactivity for stomach ailments by *E. transvaalense* and *E. croceum* bark can at least be explained by the presence of these phenolic compounds and tannins (Van Wyk *et al.*, 2005).

2.4.2 *Euclea undulata*

Euclea undulata Thunb. (Ebenaceae) common names: guarrie (Khoi); common guarri (E); gewone ghwarrie (A); umgwali (Xhosa); mokoerekoere (Tswana); gwanxe, inkunzane; umshekizane; umbophanyamazane (Z); inhlangu (Swati); chizuzu (Shona); mokwere kwere (Sotho). Plant parts used: roots.

2.4.2.1 Description

This is a dense, erect, twiggy, evergreen dioecious shrub or small tree. The leaves are alternate or arranged in pseudo-whorls and crowded at the ends of the branches. The leaves are small, obovate to narrow elliptic, leathery, yellowish green to dark green or blue-green above and paler green below, sometimes rustic-brown due to glands dotted over the surface. The flowers are small and whitish in auxiliary raceme-like sprays up to 2 cm long. The fruit is spherical, thinly fleshed, reddish brown becoming black when mature (Coates Palgrave, 1984). The species is widespread occurring on rocky slopes in all the provinces except the Free State; *Euclea undulata* var. *undulata* occurs from Worcester in the Western

Cape to Komga in the Eastern Cape while *E. undulata* var. *myrtina* (small leaved guarrie) is found in Namibia, Limpopo and into KwaZulu-Natal, Mpumalanga and Swaziland.

2.4.2.2 Medicinal uses

The plant is an old-fashioned Cape remedy for heart diseases and the powdered bark is a Southern Sotho headache remedy. An infusion of the root bark is said to be a purgative. It is also reported that the root is used in South Africa as a remedy for toothache and other pains (Watt and Breyer-Brandwijk, 1962). Leaf preparations are taken orally in the Western Cape to treat diarrhoea and disorders of the stomach, and as a gargle to relieve tonsillitis. Elsewhere in the country root infusions are used as an enematic or as an ingredient of *inembe* (medication taken regularly during pregnancy to ensure a trouble-free confinement). Root preparations are used to induce emesis or purgation (South African National Biodiversity Institute, 2005). Herbalists and traditional healers in the Venda region of Limpopo make use of *E. undulata* to treat diabetes (Dr. Emmanuel Tshikalange, Department of Plant Science, University of Pretoria, Pretoria, 0002, South Africa, personal communication).

2.4.2.3 Phytochemistry/bioactivity

Earlier studies on the phytochemistry of *Euclea* species have identified triterpenoids and aliphatics in the branches and leaves (Costa *et al.*, 1978) and naphthoquinones in the root, stem and fruit (Van der Vyver and Gerritsma, 1973, 1974). In another study, the naphthoquinones 7-methyl-juglone and diospyrin were isolated from the roots and isodiospyrin from the fruits of *E. undulata* var. *myrtina*. Stems appeared to be devoid of naphthoquinones. The leaves were not included in the latter survey. Chemical tests indicated the presence (in leaves and stems) of tannins, saponins and reducing sugars, but not of alkaloids or anthraquinones or cardiac glycosides. The bark is reported to contain 3.26% tannin (South African National Biodiversity Institute, 2005).

In vitro antimicrobial activity against *Staphylococcus aureus* was demonstrated by aqueous extracts prepared from dried leaf material, at a concentration of 40 mg/ml. This result, together with the demonstrated presence of tannins in the leaves of this species, supports its use as an anti-diarrhoeal and for the relief of tonsillitis. No activity against *Pseudomonas*

aeruginosa, *Candida albicans* or *Mycobacterium smegmatis* was found in the preliminary tests (South African National Biodiversity Institute, 2005).

As both anti-diarrhoeal and purgative actions are reported for this species, dosage and method of preparation require standardisation. Its use as an anti-diarrhoeal by pregnant women and children is not recommended.

2.4.3 *Euclea natalensis*

Euclea natalensis A.DC. (Ebenaceae); common names: Natal guarri (E); Natalghwarrie (A); mutanqule (V). Plant parts used: bark and root (Van Wyk and Van Wyk, 1997).

2.4.3.1 Description

This is a shrub or small to medium-sized tree with a somewhat spreading crown. The leaves are elliptic to obovate-oblong, tough and leathery, glossy dark green above and densely covered with pale rusty woolly hairs below. The flowers borne in dense, branched, auxiliary heads, are small greenish white to cream in colour and sweetly scented. The fruit is a spherical red berry becoming black when mature (Van Wyk and Van Wyk, 1997). It occurs from coastal dune bush to about 1 000 m above sea level in a variety of habitats from dry arid areas to open woodland and riverine fringes. It is also common among rocks and on koppies (Coates Palgrave, 1984).

2.4.3.2 Medicinal uses

An infusion of *Euclea natalensis* is used by the Zulu people as a purgative and for abdominal complaints, but is liable to produce emesis. The plant is thought to be poisonous but is an ingredient in a Zulu scrofula remedy (Watt and Breyer-Brandwijk, 1962). Among the Shangaan the charred and powdered root is applied to skin lesions in leprosy and taken internally for ancylostomiasis. The Tshonga apply the powdered root for the relief of toothache and headaches (Watt and Breyer-Brandwijk, 1962). Van Wyk and Van Wyk (1997) also reported its medicinal usage. The twigs are used as toothbrushes. In the Venda region roots of *E. natalensis* are used to treat diabetes by herbalists and traditional healers

(Dr. Emmanuel Tshikalange, Department of Plant Science, University of Pretoria, Pretoria, Pretoria, 0002, South Africa, personal communication).

2.4.3.3 Phytochemistry/bioactivity

According to the literature, the following compounds were isolated from *E. natalensis*: natalenone (Ferreira *et al.*, 1977); diospyrin, 7-methyl-juglone, euclein (Van der Vyver, 1974; Lall and Meyer, 1999, 2001); isodiospyrin, mamegaquinones, BN-quinones, 8,8-dihydroxy-4,4,4-trimethoxy-6,6-dimethyl-2,2-bisnaphthyl-1,1-quinone (Tannock, 1973) and 4,8-dihydroxy-6-methyl-1-tetralone (Khan, 1985). According to Lall and Meyer (2001), the naphthoquinones present in *Euclea natalensis* was found to have inhibitory activity against *Mycobacterium tuberculosis*.

2.4.4 *Lannea edulis*

Lannea edulis (Sond.) Engl. (Anacardiaceae); common names: wild grape (E); wildedruif (A); pheho (Ts); muporotso (V); mutshutsuhgwa (V). Plant parts used: bark of the woody underground rootstock.

2.4.4.1 Description

This is a small shrublet of up to 1 m in height, with short, leafy branches developing from an underground rootstock. The compound leaves are densely hairy, particularly on the lower side. Small yellowish flowers are borne in erect clusters, followed by numerous small red to purplish-black fleshy berries (Van Wyk and Malan, 1988). It is widely distributed in the grassland areas of the summer rainfall region of South Africa (Van Wyk *et al.*, 2005).

2.4.4.2 Medicinal uses

Decoctions or infusions of the root bark are used to treat diarrhoea. Leaf poultices or leaf infusions are sometimes applied externally to treat sore eyes, boils, and abscesses (Van Wyk *et al.*, 2005). Herbalists and traditional healers in Venda use *L. edulis* to treat

diabetes (Dr. Emmanuel Tshikalange, Department of Plant Science, University of Pretoria, Pretoria, 0002, South Africa, personal communication).

2.4.4.3 Phytochemistry/Bioactivity

Little is known about the chemistry or bioactivity of *L. edulis*, however, the bark has been found to be rich in phenolic compounds and tannins (Van Wyk *et al.*, 2005).

2.4.5 *Spirostachys africanus*

Spirostachys africanus Sond. (Euphorbiaceae); common names: tamboti (E); tambotie (A); muonze (V). Plant parts used: bark.

2.4.5.1 Description

This is a medium-sized deciduous tree with a rounded crown. The leaves are ovate to elliptic with two minute blackish glands at the junction with the petiole (van Wyk and Van Wyk, 1997). It contains a milky latex and the sexes are separated on the same plant. The flowers are very small and are produced in the axils of distinctive reddish bracts in slender catkin-like spikes (Coates Palgrave, 1984). The bark is dark grey to blackish and cracked in a grid-like pattern. It is found in the Bushveld, usually at low altitudes, on heavy soils along rivers and streams.

2.4.5.2 Medicinal use

Inhalation of the smoke causes headaches and nausea, and food directly exposed to the smoke is said to be poisonous. The latex is toxic and may cause skin irritation (Van Wyk and Van Wyk 1997). Although no evidence has been found in the literature on the plant's use by the Venda traditional healers for diabetes it has been personally communicated that a decoction made from the stem bark is used to treat diabetic patients (Dr. Emmanuel Tshikalange, Department of Plant Science, University of Pretoria, Pretoria, 0002, South Africa)

2.4.5.3 Phytochemistry/bioactivity

The latex from the wood of tamboti contains diterpenes, such as stachenone, stacenol and other structurally related acid metabolites and diosphenols (Van Wyk *et al.*, 2002)

2.4.6 *Schkuhria pinnata*

Schkuhria pinnata (Lam.) Cabrera (Asteraceae); common names: dwarf marigold (E); kleinkakiebos (A); canchalagua, escobilla, vassourinha, (Spanish); gakuinini (Kikuyu). Plant parts used: whole plant.

2.4.6.1 Description

Schkuhria pinnata is a small, herbaceous plant, which was introduced into South Africa from the mountainous regions of South America (Taylor, 2006). It is a common highly branched annual herb up to 60 cm tall with deep and finely divided leaves. Flowerheads are borne in branched, flat-topped inflorescences. The disc and ray florets are yellow. This common weed was first recorded in South Africa in 1898, when British soldiers introduced it with imported fodder for their horses (Bromilow, 1995). It occurs in cultivated lands, gardens, along roadsides, in overgrazed grasslands and wastelands (Grabandt, 1985). In South America it grows in remote places, in valleys and on slopes at 2,000 to 3,000 m above sea level (Taylor, 2006).

2.4.6.2 Medicinal uses

Andean healers (South America) recommended its use as a way to improve and support healthy looking skin. Topically it is being used as a skin tonic, for blackheads, pimples, eye wash, wound wash, insect bites and swellings (Taylor, 2006). Traditionally, a mild bitter tonic is prepared which is used as a blood cleanser and kidney tonic. *S. pinnata* is also used for leprosy, swelling, respiratory problems, bronchitis, fever, throat problems, as an aphrodisiac, for menstrual problems, stomach problems, headaches and as a strong anti-spasmodic. Furthermore, it is said to be excellent for hypertension and nervousness, for diarrhoea, venereal disease, as a diuretic, and for the treatment of diabetes. It is used to promote and support normal metabolism and blood cleansing, which can also lead to overall

improvement of the skin, as it is regarded as tonic for both the liver and kidneys (Taylor, 2006).

It is considered to be anti-diarrhoeal and anti-emetic. Antiseptic leaf decoctions are used for wounds and fever. Mixed with maternal milk, it is used as an anti-emetic for infants. Leaf decoctions are also used in antipyretic baths and in poultices for migraine and as a tea for pain and swelling. Brazilians add powdered root to bathwater when "cleaning their blood". They apply strained leaf juice for eye ailments and to infected wounds (erysipelas). A tea can be made by steeping one or more teaspoons of dried plant material per cup. *S. pinnata* is used by traditional healers and herbalists in the Ga-Rankuwa area, Gauteng to treat diabetes (Sr. Lia Matibe, Medical University of South Africa, Pretoria, 0002, South Africa, personal communication).

The common names escobilla, chanchalagu and vassourina are used for the description of concoctions of two different plant species from two separate families, *S. pinnata* and *Scoparia dulcis* L. In the literature it is not clear exactly which concoction from which plant is used for which specific ailment and if both are used for all the above mentioned ailments (Taylor, 2006).

According to Watt and Breyer-Brandwijk (1962), the powdered leaf of *S. pinnata* is swallowed with water as a remedy for malaria, influenza and for colds. The Kikuyu name implies "one with quinine", probably due to the bitter taste. It plays a significant role in the treatment of malaria in central Kenya.

2.4.7 *Pteronia divaricata*

Pteronia divaricata (P.J.Bergius) Less. (Asteraceae); common names: geel gombos, geelknopbos, spalkpenbos (A). Plant parts used: whole plant.

2.4.7.1 Description

This is a twiggy, dense shrublet up to 1 m tall. The leaves are round, elliptic and velvety. Six or seven large yellow disc flower heads are clustered at the ends of the branchlets,

flowering from September to November. It occurs on sandy and rocky soils from Namibia to Hopetown in the Northern Cape (Manning and Goldblatt, 2000).

2.4.7.2 Medicinal uses

In the literature, no evidence was obtained of the medicinal uses of *Pteronia divaricata*, although some *Pteronia* species have been reported to be toxic to livestock, e.g., *P. pallens* L.f. (Van Wyk *et al.*, 2000) and others do have medicinal value such as *P. camphorata* (L.) L., *P. onobromoides* DC., and *P. stricta* Aiton (Watt and Breyer-Brandwijk, 1962). *P. divaricata* is being used to treat diabetes in the Clanwilliam area, Western Cape (Mr Peter Maltz, Traditional Health Practitioner, 96 Arum Road, Kommetjie, Westren Cape, South Africa, personal communication).

2.4.8 *Ziziphus mucronata*

Ziziphus mucronata Willd. (Rhamnaceae); common names: buffalo-thorn (E); blinkblaarwag-‘n-bietjie (A); mokgalo (North Sotho; Ts); umphafa (Xh, Z); umlahlankosi (Z). Plant parts used: leaves.

2.4.8.1 Description

This is a small shrub to medium-sized tree. The leaves are ovate to broadly ovate, glossy dark green above and the lower surface slightly hairy. The leaf base is markedly asymmetric and the margin finely toothed over the upper two-thirds. The stipules are spinescent, one hooked, the other straight, or the plant is unarmed. Flowers are in axillary clusters and are small, yellowish green. The fruit is a sub-globose drupe, shiny reddish to yellow brown (Van Wyk and Van Wyk, 1997). These trees occur in a wide variety of habitats throughout South Africa.

2.4.8.2 Medicinal uses

According to Coates Palgrave (1984) it is used as a remedy for pain, as a poultice for the treatment of boils and other skin infections as well as for the treatment of tubercular gland swellings. It is also used by sufferers of dysentery and lumbago. The Zulu use it for the

relief of chest pains and coughs (Coates Palgrave, 1984; Van Wyk and Van Wyk, 1997). Warm infusions of the root, bark or leaves are taken orally as tea or decoctions are used topically to treat sores, boils and swelling (Van Wyk *et al.*, 2005). According to Mushtaq (2007), 4–5 fresh leaves of *Ziziphus jujube* Miller are plucked, washed and chewed daily by diabetics in the Attock district of Pakistan to lower blood glucose levels. According to Ms. G. Masuku, a local herbalist in the Pilanesberg area, North West (Ms Grace Masuku, Herbalist P.O. Box 377, Saulsport, 0318, North West, South Africa, personal communication), a tea is prepared from the leaves of *Ziziphus mucronata* combined with powdered material from *Viscum* species for the treatment of diabetes.

2.4.8.3 Phytochemistry/bioactivity

Several alkaloids such as mucronine D, commonly referred to as peptide alkaloids are known from *Ziziphus* species (Van Wyk *et al.*, 2005). The alkaloid frangufoline, is a strong sedative and is structurally closely related to some of the alkaloids extracted from *Z. mucronata* (Van Wyk *et al.*, 2005).

2.4.9 Aloe ferox

Aloe ferox Mill. (Asphodelaceae);. common names: bitter aloe (E); bitteraalwyn, Kaapse aalwyn (A); umhlaba (Xh,Z,S). Plant parts used: leaves.

2.4.9.1 Description

Aloe ferox is a robust single-stemmed plant between 2 and 5 m tall. The leaves are broad and fleshy, usually a dull to greyish green with brown spines on the edges as well as on the upper and lower surfaces. The inflorescence is a raceme with bright orange-red flowers (Van Wyk and Smith, 1996). It is a widely distributed *Aloe* species occurring from the Western to the Eastern Cape as well as in southern KwaZulu-Natal and the extreme southeastern parts of the Free State. It occurs in a wide range of habitats varying from mountain slopes, rocky ridges and flat open plains (Van Wyk and Smith, 1996).

2.4.9.2 Medicinal uses

This plant is an important commercial laxative but is also used for arthritis, eczema, conjunctivitis (Watt and Breyer-Brandwijk, 1962; Bruce, 1975), hypertension and stress (Pujol, 1990). According to Van Wyk *et al.* (2005) a small crystal of the crude drug, about twice the size of a match head, is taken orally as a laxative and half the dose for arthritis. The fresh bitter sap is instilled directly for conjunctivitis and sinusitis. *A. ferox* is also used to treat diabetes according to Margaret Roberts, a well-known South African herbalist (Ms. Margarte Roberts, P.O. Box 41, DeWildt, 0251, North West, South Africa, personal communication).

2.4.9.3 Phytochemistry/bioactivity

The main purgative ingredient is anthrone C-glucoside alion (= barbalion). The wound healing properties of aloe gel are ascribed to glycoproteins. Anthraquinone derivatives act as stimulant laxatives (Bruneton, 1995).

2.4.10 *Warburgia salutaris*

Warburgia salutaris (G.Bertol.) Chiov. (Canellaceae); common names: pepperbark tree (E); peperbasboom (A); isibhaha, amazwecehllabayo (Z); mulanga, manaka (V); shibaha (Ts). Plant parts used: stem bark.

2.4.10.1 Description

This is a slender tree between 5 and 10 m tall. The bark is rough and a rich brown colour with yellow corky lenticels (Venter and Venter, 1996; Coates Palgrave, 2002). The leaves are simple and alternately arranged, elliptic to lanceolate, a dark glossy green above and a pale dull green below. The bisexual flowers can be green or white and are solitary or in flower-heads or cymes. The ten stamens are joined together to form a tube in the centre of the flower, enveloping the ovary and most of the style. The fruit is a spherical berry that turns black when mature (Coates Palgrave, 1984). It occurs in evergreen forests and wooded ravines in the northeastern areas of southern Africa (Coats Palgrave, 1984).

2.4.10.2 Medicinal uses

Warburgia is one of the most important medicinal plant species in southern Africa. The inner bark is reddish, bitter and peppery and has a variety of applications. It is used to treat common colds, to open sinuses, for chest complaints as well as against malaria (Coates Palgrave, 1984). According to Van Wyk and Gericke (2000) it is also used as a natural antibiotic, for venereal diseases, abdominal pain, constipation, in the treatment of cancer, for rheumatism, and stomach ulcers. Powdered bark decoctions and infusions are taken orally against malaria. It is also applied externally to cuts as well as on the temples for headaches (Van Wyk and Gericke, 2000). It is ground and snuffed to open sinuses, chewed or smoked and inhaled for chest complaints (Coates Palgrave, 1984). It is also used to treat diabetes (Ms. Margaret Roberts, P.O. Box 41, De Wildt 0251, North West, South Africa, personal communication)

2.4.10.3 Phytochemistry/bioactivity

The bark contains numerous drimane sesquiterpenoides, such as warburganal and polygodial, as well as tannins and mannitol (Watt and Beyer-Brandwijk, 1962; Van Wyk and Gericke, 2000). Warburganal and polygodial both showed profound anti-candidal activity (Van Wyk and Gericke, 2000). Polygodial is potentially useful in clinical medicine as an adjustment to treatment with antibiotics and antifungals which have poor membrane permeability (Iwu, 1993). Mannitol is used against dyspepsia and as a diuretic (Bruneton, 1995). According to Hutchings *et al.* (1996) drimenin has insect antifeedant properties and also showed anti-bacterial and anti-ulcer activity (Van Wyk *et al.*, 2005). Potential antifungal activity was exhibited by an isolated sesquiterpenoid dialdehyde (Hutchings *et al.*, 1996). Muzigadial, another sesquiterpenoid, is according to Rabe and Van Staden (1997, 2000) responsible for the antibacterial activity.

2.4.11 *Momordica balsamina*

Momordica balsamina L. (Cucurbitaceae); common names: balsam pear (E); laloentjie (A); mohodu (S); nkaka (Tshonga). Plant parts used: fruit.

2.4.11.1 Description

Balsam pear is a perennial creeping herb with slender stems, lobed leaves and tendrils for attachment. The solitary trumpet yellow flowers are followed by pointed fruits that turn orange to red when mature. The edible seeds have bright red arils that are also considered to be edible. Leaves and young fruit are cooked and used as vegetables (Van Wyk and Gericke, 2000). Commonly found in tree savannas in sandy soils (Van Rooyen, 2001).

2.4.11.2 Medicinal uses

Momordica balsamina is said to be effective in treating diabetes (van Wyk and Gericke, 2000; Van Rooyen, 2001). Although it is used as an anti-diabetic, careful tests do not support its use. It showed moderate hypoglycaemic action when tested in rabbits, however, there has been no definite assurance of insulin-like properties (*Momordica balsamina*, 2006). *M. balsamina* emits a strong unpleasant smell when bruised (Watt and Breyer-Brandwijk, 1962) A liniment, made by infusing the fruit (minus the seed) in olive oil or almond oil, is used as an application to chapped hands, burns and haemorrhoids and the mashed fruit is used as a poultice. An extract is administered for the relief of dropsy. The plant is much used in West Africa as a medicine in both man and horse, particularly as a bitter stomachic, as a wash for fever and for yaws, and as a purgative (Watt and Breyer-Brandwijk, 1962). The fruit pulp, mixed with oil, is used as an antiphlogistic dressing. The root is used as an ingredient in an aphrodisiac or with the seeds and fruit as an abortifacient and in the treatment of urethral discharge. Among the Pedi, the fruit is believed to be deadly poisonous and this view is supported by the report that a few drachms of it, given to a dog, is fatal, death being due to violent vomiting and purging (Watt and Breyer-Brandwijk, 1962). The Shangaan prepare tea from the leaves as a blood purifier and for liver deficiencies. In dry seasons, postnatal mothers eat the leaves to stimulate milk production (*Momordica balsamina*, 2006). One small cup of fresh *M. charantia* L. fruit juice daily is used to lower blood glucose levels in the Attock district in Pakistan. It is also taken in the form of a tea for various other ailments (Mushtaq *et al.*, 2007).

2.4.11.3 Phytochemistry/bioactivity

M. balsamina contains a bitter principle, momordocin and two resin acids. The

plant also contains a highly aromatic volatile oil, a fixed oil, carotene, a resin and two alkaloids, one of which is a saponin. Momordocin is an amaroid obtained as a crystalline powder. A leaf extract has shown positive antibacterial activity. An infusion of the plant has shown mild, but inconsistent, anti-malarial effects hence the use of this plant by the Portuguese for “paludismo” or sometimes referred to as “yellow fever tree sickness” (Mushtaq *et al.*, 2007).

2.4.12. *Kedrostis nana*

Kedrostis nana (Lam.) Cogn (Cucurbitaceae); common names: ystervarkpatat (A).

Plant parts used: underground tuber.

2.4.12.1 Description

This is a tuberous climber with annual stems that attach to their support by tendrils. The leaves are alternate, lobed and shining dark green (Manning and Goldblatt, 2000). It has a strong odour of carbon bisulphide (Watt and Breyer-Brandwijk, 1962). The greenish-yellow flowers are unisexual and borne on separate plants during February and March. The male flowers are in short clusters whereas the female flowers are solitary. The flowers are followed by fleshy, yellow-orange fruits (Manning and Goldblatt, 2000). *Kedrostis nana* is frequently found among bushes at low altitudes, especially near the sea from the Western Cape to KwaZulu-Natal (Manning and Goldblatt, 2000).

2.4.12.2 Medicinal uses

The tuber is used as a cleansing emetic, and as a laxative. An infusion mixed with honey is taken for haemorrhoids. Infusions are used for diabetes and cancer, and in low doses for diarrhoea. A decoction of baked tuber, combined with *Dicerotheramnus rhinocerotis* (L.f.) Koekemoer is taken as a contraceptive (Rood, 1994) and may have an abortifacient action, since *K. gijef* (J.F.Gmel.) C.Jeffrey has been reported to cause abortion in goats (Hutchings *et al.*, 1996). *K. nana* has been found experimentally to produce a severe type of irritant poisoning in sheep and rabbits. The runners and roots are poisonous to sheep and rabbits, producing nausea, vomiting and diarrhoea, and death after large doses from respiratory paralysis.

The early colonists of the Cape used the roots of *K. nana* var. *latiloba* as an emetic and an infusion in wine or brandy as a purgative (Watt and Breyer-Brandwijk, 1962).

2.4.13 Artemisia afra

Artemisia afra Jacq. ex. Willd. (Asteraceae); common names: umhlonyane (Xh, Z); lengana (S,Ts); als, wildeals (A); African wormwood (E). Plant parts used: leaves.

2.4.13.1 Description

This is a highly aromatic, erect, perennial shrub of up to 2 m in height. The leaves are finely-divided and have a silver-greyish green colour due to the presence of fine hairs. The flowers are inconspicuous, yellow and borne at the ends of branches in globose capitula (Hilliard, 1977). It occurs widespread in all provinces of South Africa, with the exception of the Northern Cape, also in Lesotho, Swaziland, and northwards into tropical Africa, usually in montane habitats along forest margins and streamsides (Hilliard, 1977).

2.4.13.2 Medicinal uses

Artemisia afra is one of the most widely used traditional medicines in South Africa. It is mainly used for the treatment of coughs, croup, whooping cough, influenza, fever, diabetes, gastro-intestinal disorders and intestinal worms. It is also used as an inhalation for the relief of headaches and nasal congestion, or as a lotion to treat haemorrhoids (Van Wyk *et al.*, 2005). In traditional practice a fresh leaf is inserted into the nostril to relieve nasal congestion or placed in boiling water as a steam bath for menstrual pain or after child birth. Warmed leaves may be applied externally as a poultice to relieve inflammation and aqueous infusions are administered per rectum or applied as a lotion to treat haemorrhoids (South African National Biodiversity Institute, 2006a). *A. afra* is used mainly as an aqueous decoction or an infusion applied externally or is taken orally. The extremely bitter taste can be masked by the addition of sugar or honey. An infusion may be made with two tablespoons full (7 g) of dried ground herb to which 1 l of boiling water is added. If fresh leaves are being used four tablespoons of freshly chopped leaves are infused in 1 L of

boiling water. Fresh leaves may be added to boiling water and the vapours inhaled (South African National Biodiversity Institute, 2006a).

2.4.13.3 Phytochemistry/bioactivity

Microchemical tests indicated the presence of tannins and saponins but not of alkaloids or cardiac, cyanogenic or anthraquinone glycosides. Studies done by Silbernagel *et al.* (1990) identified the triterpenes σ - and β -amyrin and friedelin as well as the alkanes ceryl cerotate and *n*-nonacosane in the leaves of the South African collections of *A. afra*. The investigation of leaf exudate flavonoids revealed the presence of two luteolin methyl ethers (Wollenweber *et al.*, 1989). Jakupovic *et al.* (1988) analyzed the sesquiterpene lactones of *Artemisia* and 10 guaianolides and 5 glaucolides were detected. Analyses of the essential oils obtained from the leaves have demonstrated considerable variation in oil composition. The major components of the oil appear to be α - and β -thujone, 1,8-cineole, camphor and α -pinene (Graven *et al.*, 1992). Also present are terpenoids of the eudesmadien- and germacratien types as well as coumarins and acetylenes (Van Wyk *et al.*, 2005)

Antihistaminic and narcotic analgesic effects have been reported following preliminary tests (Hutchings *et al.*, 1996; Van Wyk *et al.*, 2005). The volatile oil, which contains mainly 1,8-cineole, α -thujone, β -thujone, camphor and boreol, has antimicrobial and anti-oxidative properties (Graven *et al.*, 1992). Anti-malaria assays done on dried aerial parts of Tanzanian plants showed weak activity against *Plasmodium falciparum*. Fresh ethanol leaf extracts showed no activity against Leuk-L1210 and Sarcoma-WM256(IM) lines (Charlson, 1980). According to Watt and Breyer-Brandwijk (1962), *A. afra* is being used to keep urine free from sugar in the case of diabetes mellitus.

2.4.14 *Catharanthus roseus*

Catharanthus roseus (L.) G.Don (Apocynaceae); common names: Madagascar periwinkle (E); kanniedood (A); isisushlungu (Z). Plant parts used: aerial parts.

2.4.14.1 Description

This is a semi-woody evergreen perennial herb up to 900 mm in height. The leaves are opposite and a glossy dark green, with a prominent white midrib. The five petaled flowers are up to 40 mm in diameter and vary from pink to white. The flowers are tubular, with a slender corolla tube. Periwinkles are commonly grown in South African gardens, but originate from Madagascar and have become naturalized in tropical and sub-tropical regions of the world (Van Wyk *et al.*, 2005).

2.4.14.2 Medicinal uses

The plant is traditionally used in South Africa for the treatment of diabetes and rheumatism (Watt and Breyer-Brandwijk, 1962). Alkaloid extracts of the aerial parts are used to treat various forms of cancer such as breast and uterine cancer and Hodgkin's and non-Hodgkin's lymphoma (Bruneton, 1995). An infusion of the leaf is used to treat diabetes, but even diluted mixtures can be extremely toxic. The two main alkaloids of the plant are used in combined chemotherapy and small doses are injected weekly or monthly (Van Wyk *et al.*, 2005). According to Mushtaq *et al.* (2007), extracts obtained from fresh leaves are being used for diabetes in Pakistan. A small teaspoon full is taken in the morning.

2.4.14.3 Phytochemistry/bioactivity

Various alkaloids such as catharanthine, leurosine and vindoline are responsible for the hypoglycemic effect. The two binary indole alkaloids vincristine and vinblastine are used in cancer chemotherapy (Van Wyk *et al.*, 2005). The binary alkaloids prevent cell division in the metaphase by binding to the protein tubulin and blocking its ability to polymerise into microtubules (Bruneton, 1995; Van Wyk *et al.*, 2005).

2.4.15 *Cnicus benedictus*

Cnicus benedictus L. (Asteraceae); common names: karmedik (A); holy thistle (E).

Plant parts used: leaves.

2.4.15.1 Description

This is an annual herb up to 70 cm tall with a rosette of basal leaves. The lance-shaped leaves are indented with spiny edges. Yellow flowers are borne in a terminal flower head surrounded by a circle of spiny bracts (Van Wyk *et al.*, 2005). *Cnicus benedictus* is native to Europe and Asia, but was introduced to South Africa more than 150 years ago and is currently a widely distributed weed in the Cape as well as on the Highveld (Henderson and Anderson, 1966; Smith, 1966).

2.4.15.2 Medicinal uses

The plant is used as a cholagogue, stomachic and tonic (Bruneton, 1995). The recorded uses in South Africa include brandy tinctures for internal cancers, for diabetes and arthritis (Watt and Beyer-Brandwijk, 1962, Smith, 1966 and Rood, 1994). This medicine is used as an aromatic bitter to stimulate the secretion of gastric juices which increase the appetite (Van Wyk *et al.*, 2005). The use of *Cnicus benedictus* for bacterial infections, indigestion and flatulence as well as for viral infections has been investigated but the scientific evidence to substantiate its healing properties is still lacking. It is also used as a contraceptive, as an appetite stimulant, an astringent, for bleeding, as a blood purifier, for boils, colds as well as in the treatment of cancer, heart and liver ailments and malaria (Basch *et al.*, 2006). Boiling water is poured over 1.5 to 3g of the ground dried herb, steeped for 10 to 15 min to prepare a tea and then taken three times daily (Basch *et al.*, 2006). A cup of the unsweetened infusion is taken half an hour before meals (Van Wyk *et al.*, 2005).

2.4.15.3 Phytochemistry / bioactivity

A bitter sesquiterpenoid lactone, cnicin, is probably the main active ingredient (Bruneton, 1995). Lignan lactones, such as trachelogenin, contribute to the bitterness of the plant. The plant also contains volatile oil with the terpenoids *p*-cymene, fenchone, and citral and the aromatic substances cinnamaldehyde and benzoic acid all of which can contribute towards the pharmacological activity of the plant (Van Wyk *et al.*, 2005).

2.16 *Psidium guajava*

Psidium guajava L. (Myrtaceae); common names: guava (E); koejawel (A); ugwawa (Z).

Plant parts used: leaves.

2.16.1 Description

This is an evergreen shrub or small tree, 2 to 5 m tall (Henderson, 2001). The bark peels off in flakes, revealing the characteristic smooth trunk. The large bronze, turning to light green, ovate to oblong-elliptic leaves are borne opposite each other. The veins are conspicuously impressed above and raised below. Small white flowers with numerous stamens are produced in groups of 1-3 during October to December, followed by rounded or pear-shaped many-seeded berries. Guavas are an important commercial crop due to their delicious taste and high vitamin C content. The guava occurs naturally in tropical America up to Peru, but has become naturalized in many parts of the world. It is found as a weed in the warm subtropical areas of KwaZulu-Natal, Mpumalanga and Limpopo in South Africa (Van Wyk *et al.*, 2005) and is currently classified as a category 2 invasive weed in South Africa.

2.4.16.2 Medicinal uses

Guava leaves are commonly used in South Africa as a remedy for diarrhoea (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996). The leaves are also used for several other ailments including diabetes, fever, coughs, ulcers, boils and wounds (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996). The main ethnotherapeutic use in Africa is said to be for malaria (Iwu, 1993). Crushed leaves are boiled in water and the infusion is either taken orally as a tea or as an enema (Hutchings *et al.*, 1996). A common use in Pakistan is to make a hot water extract from dried guava leaves to reduce blood glucose levels in diabetes (Mushtaq *et al.*, 2007).

2.4.16.3 Phytochemistry/bioactivity

Numerous tannins and phenolic compounds have been identified from *Psidium guajava* of which the glycoside of ellagic acid, amritoside, is of particular importance. Other

biologically interesting compounds are guajaverin and arabinopyroside of quercetin. The leaves contain essential oils and triterpenoids (Anonymous, 1996). Ellagic acid, is a known intestinal astringent and haemostatic (Anonymous, 1996). Bruneton (1995) explains the therapeutic value of the plant against diarrhoea and dysentery. The tannins are of value because of their vasoconstricting effects and their ability to form a protective layer on the skin and mucous membranes (Bruneton, 1995). These effects, together with proven antibacterial and antifungal activity, result in the effective treatment of both internal and external infections (Bruneton, 1995). Quercetin contributes to the efficacy of the medicine, because it is a known antioxidant with anticarcinogenic, anti-HIV and antibiotic effects (Anonymous, 1996).

2.4.17 *Terminalia sericea*

Terminalia sericea Burch. ex. DC. (Combretaceae); common names: silver cluster-leaf (E); vaalboom (A); mogonono (T); moxonono (NS); mususu (Sh, V); amangwe (Z); mangwe (Ndebele). Plant parts used: stem bark.

2.4.17.1 Description

It is a small to medium-sized deciduous tree up to 8 m tall, with a single erect trunk and wide spreading canopy (Coates Palgrave, 1984; Van Wyk and Van Wyk, 1997; 2000). The bark is thick, fibrous, dark grey and deeply longitudinally fissured. The leaves are clustered towards the ends of the branches, narrowly obovate-elliptic and densely covered with silky, silvery hairs (Van Wyk *et al.*, 2000). Small pale cream flowers are borne in axillary spikes and have an unpleasant smell. The fruit is surrounded by two broad papery wings, and are pink to purplish red when mature (Van Wyk *et al.*, 2000). These trees are normally found in the Bushveld, on deep sandy soils, often in dense stands (Van Wyk *et al.*, 2000).

2.4.17.2 Medicinal uses

Root decoctions are used as a traditional Tswana remedy for stomach disorders and diarrhoea (Watt and Beyer-Brandwijk, 1962; Coates Palgrave, 1984; Hutchings *et al.*, 1996). Decoctions of the roots, that have a very bitter taste, are taken to cure diarrhoea, to

relieve colic but are also used as an eye wash. Hot root infusions are used for the treatment of pneumonia (Coates Palgrave, 1984) whereas the bark is taken for diabetes (Watt and Beyers-Brandwijk, 1962). Decoctions and infusions are taken orally or applied externally. Ground bark eaten with maize meal is taken for diabetes (Van Wyk *et al.*, 2005).

2.4.17.3 Phytochemistry/bioactivity

Several pentacyclic triterpenoids have been isolated from *Terminalia* species (Buckingham, 1996), of which sericic acid and an ester thereof, known as sericoside, are the main compounds in the roots (Bombardelli *et al.*, 1974). The medicinal activity of the Combretaceae family is mainly ascribed to stilbenoids, triterpenoids, and saponins (Rogers, 1996). Triterpenoids and saponins are well known for their antimicrobial and anti-inflammatory activity. The antidiarrhoeal effects may be due to tannins (Bruneton, 1995).

2.4.18 *Sutherlandia frutescens*

Sutherlandia frutescens (L.) R.Br. (Fabaceae); common names: kankerbos, gansies (A); cancer bush (E). Plant parts used: aerial parts.

2.4.18.1 Description

This is an attractive small shrub up to 1.2 m high. The compound leaves are greyish green, slightly to densely covered with hair giving it a silvery appearance. The characteristic red pea family flowers are followed by bubble-like, duck shaped pods giving rise to the Afrikaans common name “gansies”. *Sutherlandia frutescens* is a small genus consisting of five members, mainly restricted to southern Africa with representatives in South Africa, Botswana and Namibia where they are widely distributed in the dry areas of the Western and Northern Cape, often in disturbed places.

2.4.18.2 Medicinal uses

S. frutescens is used internally for the treatment of cancer, gastric ailments, gynaecological problems, rheumatism, oedema and fevers and also as a bitter tonic or blood purifier. According to tradition and folklore the plant’s uses include remedies for colds, influenza, chicken-pox, diabetes, varicose veins, piles, inflammation, liver problems, backache and

rheumatism. Externally it is used to treat eye infections and wounds and as a douche for the prolapse of the uterus.

Approximately 10 g (\pm 3 tablespoons full) of dried ground herb is infused in 1 L of boiling water, strained and when cold taken in half a teacupful dose (90 ml) three-times daily. Children 6-12 years are given 45 ml (South African National Biodiversity Institute, 2006b).

2.4.18.3 Phytochemistry/bioactivity

The plant is rich in amino acids and pinitol, but has small quantities of saponins and no alkaloids, according to current biosystematic and chemosystematic studies done (Van Wyk *et al.*, 2005). The non-protein α -amino acid canavanine has been detected in the seeds of the species (Bell *et al.*, 1978). Studies using 50% ethanol extracts of the fresh flowers of *Sutherlandia frutescens* found no antitumour activity against CA-Lewis lung, Leuk-L 1210 or Sarcoma 180 solid tumours in mice. Similar extracts, assayed for cytotoxicity against CA-9KB cell lines, at a concentration of 2 μ g/ml, proved to be inactive (Charlson, 1980). *S. frutescens* seeds contain canavanine that has according to Southon (1994) antitumourigenic properties and it is possible that this, or some other amino acid, is responsible for the reported benefits in treating cancer (Van Wyk *et al.*, 2005). No *in vitro* antimicrobial activity against *Pseudomonas aeruginosa*, *Candida albicans* or *Mycobacterium smegmatis* was observed. Some activity was recorded against *Staphylococcus aureus* (South African National Biodiversity Institute, 2006b). The presence of pinitol, however, explains the traditional anti-diabetic use (Van Wyk *et al.*, 2005).

2.4.19 *Bridelia micrantha*

Bridelia micrantha (Hochst.) Baill. (Euphorbiaceae); common names: coastal goldenleaf; mitzeerie/mzerie, wild coffee (E); bruinstinkhout (A); mitserie (A); incinci, isihlalamangewibi, isihlalamangwibi, umhlahle, umshonge, umhlalamagwababa, umhlalamgwababa, umhlalimakwaba, umhlalamkhwaba (Z). Plant parts used: stem bark.

2.4.19.1 Description

This is a medium to large deciduous tree with a spreading crown characterised by scattered bright red leaves, while the rest of the leaves are glossy dark green above but paler green below. The venation of the leaves is prominent in a herringbone pattern. The very small flowers are borne in axillary clusters and are yellowish green. Flowers are followed by small edible black berries (Van Wyk and Van Wyk, 1997). The bark is brown to grey, slightly flaking and rough in mature specimens (Coates Palgrave, 2002). It occurs in coastal, riverine, and swamp forest, usually in moist places (Van Wyk and Van Wyk, 1997).

2.4.19.2 Medicinal uses

In southern Africa the stem bark is used as an expectorant, as a laxative, and in the therapy of diabetes (Iwu, 1993). Powdered bark is applied topically to burns, which reputedly enhances the rate of healing (Venter and Venter, 1996). The Venda also use it to treat wounds, burns, toothache and venereal diseases (Mabogo, 1990). According to Hutchings *et al.* (1996) the Zulu people take an infusion as an emetic.

2.4.19.3 Phytochemistry/bioactivity

The following compounds were isolated from *Bridelia micrantha* (Pegel and Rogers, 1968 cited in Hutchings *et al.*, 1996): epifreidelinol, taraxol, gallic acid and ellagic acid. Gallic acid and ellagic acid seem to have antifungal and antiviral properties. Gallic and ellagic acid are antioxidants and were found to show cytotoxicity against cancer cells (Phytochemicals, 2007ab). Gallic acid is used as a remote astringent in cases of internal haemorrhage as well as in the treatment of albuminuria and diabetes.

2.4.20 *Sclerocarya birrea*

Sclerocarya birrea (A.Rich.) Hochst. (Anacardiaceae); common names: marula, cider tree (E); maroela (A); umganu (Z); morula (NS). Plant parts used: roots, bark, and leaves.

2.4.20.1 Description

This is a medium to large deciduous tree with an erect trunk and rounded, spreading crown. The leaves consists of 3 to 7 pairs of leaflets with a terminal one, dark green above and a paler bluish green below (Van Wyk and Van Wyk, 1997). The flowers are borne in small,

oblong clusters. Male and female flowers occur separately, usually, but not always on separate trees, before the new leaves. The flowers are small, with red sepals and yellow petals. The rough bark is flaky, with a mottled appearance due to contrasting grey and pale brown patches (Van Wyk *et al.*, 2005). A watery latex is present (Van Wyk *et al.*, 2000). An almost spherical fleshy fruit is borne in late summer to mid-winter, ripening to yellow after falling to the ground. The stone is hard with two to three openings plugged by lids. The tree is widely distributed throughout South Africa in bushveld and woodland (Van Wyk *et al.*, 2005).

2.4.20.2 Medicinal uses

Sclerocarya birrea is being used for diarrhoea, dysentery and unspecified stomach problems. It is also being used to combat fever and in the treatment of malaria (Watt and Breyer-Brandwijk, 1962; Hutchings, 1989; Hutchings *et al.*, 1996). Pujol (1990) reported that it is used as a general tonic, for indigestion and for the treatment of diabetes. Decoctions or leaf infusions are taken for diabetes (Iwu, 1993).

2.4.20.3 Phytochemistry/bioactivity

According to Galvez *et al.* (1993) the bark contains procyanidins whereas Watt and Breyer-Brandwijk (1962) and Iwu (1993) reported that it contains gallotannins, flavonoids and catechins. No detailed information has been documented. The antidiarrhoeal effects have been experimentally linked to the procyanidins (Galvez *et al.*, 1993). Claims have also been made that it possesses hypoglycaemic effects (Iwu, 1993).

2.4.21 *Brachylaena discolor*

Brachylaena discolor DC. (Asteraceae); common names: coast silver oak (E); kusvaalbos(A); muakawura, mupasa (Sh); iphahla, umpahla (Z). Plant parts used: leaves.

2.4.21.1 Description

An evergreen shrub or small tree usually 4 to 10 m in height. The bark is rough, dark grey to brownish-grey. The leathery leaves are lanceolate to obovate, dark green above and pale whitish below and covered with dense hairs. The leaf margin is entire or obscurely and

irregularly toothed. The sexes are on different plants. The flowerheads are grouped in terminal panicles with creamy-white individual flowers. The fruit is a small achene, tipped with a tuft of bristly hairs (Coates Palgrave, 1984; Van Wyk and Van Wyk., 1997). It occurs in coastal woodland and bush as well as littoral scrub and on the margins of evergreen forests (Coates Palgrave, 1984).

2.4.21.2 Medicinal uses

According to Watt and Breyer-Brandwijk (1962) this species is being used for diabetes, renal conditions and as a tonic. Leaf infusions are used by the Zulu in the treatment of diabetes (Venter and Venter, 1996) and for intestinal parasites and for round worms (Watt and Breyer-Brandwijk, 1962).

2.4.21.3 Phytochemistry/bioactivity

Onopordopicrin has been isolated from aerial parts of *Brachylaena discolor* (Hutchings *et al.*, 1996). Cytotoxic, antibacterial, and antifungal activities have been reported for onopordopicrin (Lonergan *et al.*, 1992).

2.4.22 *Brachylaena elliptica*

Brachylaena elliptica (Thunb.) DC. (Asteraceae); common names: bitterleaf (E); bitterblaar (A); isiduti (X); iphahle, uhlunguhlungu (Z). Plant parts used: leaves.

2.4.22.1 Description

This is a shrub or small tree up to 4 m tall with a light grey to brown bark that becomes rough with age. The leaves are lanceolate, elliptic to ovate, dark green above and white felted below. The leaf margin is irregularly toothed and often with 2 lobes near the base giving the leaf a 3-lobed effect. The creamy white flowers are born in terminal and axillary flowerheads. The fruit is a small achene, tipped with a tuft of bristly hair (Coates Palgrave, 1984; Van Wyk and Van Wyk, 1997). *Brachylaena elliptica* occurs in bushveld on rocky outcrops and along coastal margins (Van Wyk and Van Wyk, 1997).

2.4.22.2 Medicinal uses

The leaves, which are extremely bitter tasting, are used medicinally (Van Wyk and Van Wyk, 1997) and valued by the Xhosa and Zulu as a treatment for diabetes. An infusion serves as a gargle and mouthwash (Coates Palgrave, 1984).

2.4.22.3 Phytochemistry/bioactivity

Watt and Breyer-Brandwijk (1962) stated that a *B. elliptica* infusion is bitter, contains one or more glucosides, probably no resin, and tested negative for alkaloids. It was also found that the leaf contains mucilage, tannin and a bitter ingredient which may be an alkaloid (Watt and Breyer-Brandwijk, 1962). Research has shown that infusions have no effect upon carbohydrate metabolism and little or no improvement in glycosuria or blood sugar percentages (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996). The benefit derived from its local use as a gargle arises from the demulcent and astringent effects of the mucilage and the tannins, respectively (Watt and Breyer-Brandwijk, 1962).

2.4.23 *Brachylaena ilicifolia*

Brachylaena ilicifolia (Lam.) E. Phillips & Schweick. (Asteraceae); common names: small bitter leaf (E); fynbitterblaar (A). Plant parts used: leaves.

2.4.23.1 Description

This is a shrub or small tree between 3 and 4 m in height with a grey to brown bark. The leaves are often on short lateral branches, small, narrowly oblong, lanceolate to ovate, green above and covered with whitish-green hairs below. The leaf apex is rounded with a sharp spine-like tip, the margin entire or with small teeth. The flowerheads are thistle-like and the individual flowers are cream to yellow and grouped into a capitulum. The flowers give rise to a small achene with whitish bristly hairs (Coates Palgrave, 1984). *Brachylaena ilicifolia* occurs in bush, scrub forest and on rocky hillsides (Coates Palgrave, 1984).

2.4.23.2 Medicinal uses

The leaves, which are intensely bitter, are used by Africans to treat diabetes (Coates Palgrave, 1984). It may be used in the same way as *B. elliptica* (Watt and Breyer-Brandwijk, 1962).

2.4.23.3 Phytochemistry/bioactivity

In the literature no information could be found on the phytochemistry or bioactivity of *B. ilicifolia*.

2.4.24 *Bulbine latifolia*

Bulbine latifolia (L.f.) Spreng. var. *latifolia* (Asphodelaceae); common names: broad-leaved Bulbine (E); ibhucu (Z); rooiwortel, geelkopieva (A); incelwane (Xh). Plant parts used: fresh leaves and roots.

2.4.24.1 Description

This is a perennial with tuberous roots. The leaves are thick fleshy, bright green to yellow and arranged in a rosette. The inflorescence is borne on a long stem in a cluster consisting of yellow flowers. It occurs widespread in hot dry areas in the eastern and northern parts of South Africa (Pooley, 1998).

2.4.24.2 Medicinal uses

The sap of *Bulbine* species is widely used for the treatment of wounds, burns, rashes, itches, ringworm, cracked lips (Watt and Breyer-Brandwijk, 1962; Rood, 1994; Pujol, 1990) and Herpes (Van Wyk *et al.*, 2005). Root infusions of *Bulbine latifolia* are taken orally to quell vomiting and diarrhoea (Pujol, 1990), convulsions, venereal diseases, diabetes, urinary complaints, rheumatism and blood disorders (Van Wyk *et al.*, 2005; Pooley, 1998). Leaf sap is applied directly to the skin or in the form of a warm poultice. An infusion of the roots or sometimes a brandy tincture is taken two or three-times daily (for internal use) (Watt and Breyer-Brandwijk, 1962).

2.4.24.3 Phytochemistry/ bioactivity

The stems and roots of *Bulbine* species contain anthraquinones such as chrysophanol and kinpholone (Van Staden and Drewes, 1994; Van Wyk *et al.*, 1995), but these compounds are probably of minor importance in the healing of wounds. Chrysophanol has been shown to have antibacterial properties (Bruce, 1975). The healing effect is likely to be due to glycoproteins such as, aloctin A and B, in the leaf gel (Suzuki, 1981).

2.4.25 *Carpobrotus edulis*

Carpobrotus edulis (L.) L. Bolus (Mesembryanthemaceae); common names: suurvy, perdevy, vyerank (A); gaukum (K); t’kobovy (Nama); sour fig (E). Plant parts used: leaf juice and leaf pulp.

2.4.25.1 Description

A creeping succulent perennial with light green leaves often tinted red. The long leaves are slightly bent, tapered to the apex to give a more or less triangular shape. The large yellow flowers change to pink as they mature (Le Roux, 2005). The fragrant fruit contains a jellylike, sweet-sour pulp with a multitude of small, brown seeds (Van Wyk *et al.*, 2005). Originally found in sandy, dry riverbeds along the coastlines of Namaqualand and south and eastwards along the coastline of the Western and Eastern Cape (Le Roux, 2005).

2.4.25.2 Medicinal uses

Juice from the leaves is gargled to treat throat and mouth infections (Rood, 1994). It is also taken orally for dysentery, digestive ailments, tuberculosis and as a diuretic (Watt and Breyer-Brandwijk, 1962). It is highly astringent and is applied externally to treat eczema, wounds and burns (Watt and Breyer-Brandwijk, 1962; Rood, 1994). It is also said to be effective against toothache, earache and oral and vaginal thrush. The fresh juice is taken orally or gargled whereas the leaf pulp is applied to the skin to treat wounds and infections (Watt and Breyer-Brandwijk, 1962; Rood, 1994). *C. edulis* has traditionally been used in South Africa for the treatment of diabetes mellitus (Van Huyssteen, 2003).

2.4.25.3 Phytochemistry/bioactivity

The beneficial medicinal effects are probably due to the presence of tannins. Tannins have the ability to form complexes with proteins, such as digestive enzymes and fungal or viral toxins. In addition to their antiseptic activity, tannins have a vasoconstricting effect and reduce fluid loss from wounds and burns, thereby enhancing tissue regeneration (Bruneton, 1995). The juice is said to be mildly antiseptic and highly astringent. The leaves also contain malic and citric acid (Watt and Breyer-Brandwijk, 1962).

2.4.26 *Chironia baccifera*

Chironia baccifera L. (Gentianaceae); common names: aambeibossie, bitterbossie, agdaegeneesbossie, perdebossie (A); Christmas berry, Wild Gentian (E). Plant parts used: whole plant.

2.4.26.1 Description

This is a much branched shrublet between 0.5 and 1m tall. The stems are rigid, angled or narrowly winged. The narrow, thin or slightly fleshy leaves are semiclasped at the base with a hooked tip. Solitary pink flowers with conspicuous yellow anthers are borne terminally from August to February (Pooley, 1998). The flowers are followed by bright red berries when ripe (Van Wyk *et al.*, 2005). This shrublet is found from the Cape Peninsula northwards to the Kamiesberg and eastwards into the Eastern Cape and KwaZulu-Natal. It usually grows in dry, sandy soils in the shade of other plants as well as in full sun (Van Wyk *et al.*, 2005; Dyer *et al.* 1963).

2.4.26.2 Medicinal uses

Chironia baccifera is traditionally used by the Khoi as a purgative and for the treatment of boils (Watt and Breyer-Brandwijk, 1962). It is also used in traditional medicine as a purgative and for the treatment of haemorrhoids (Watt and Breyer-Brandwijk, 1962; Smith, 1966; Rood, 1994; Pooley, 1998). A decoction of the whole plant is taken as a blood purifier to treat acne, sores and boils (Van Wyk *et al.*, 2005). Infusions may be used as a remedy for diarrhoea or for leprosy (Watt and Breyer-Brandwijk, 1962). According to Van

Wyk and Gericke (2000), the plant is used as a bitter tonic and infusions and tinctures from the leaves and stems are used to treat diabetes. Decoctions, tinctures or infusions are taken, but the plant is potentially toxic therefore use should be controlled. Plant material is fried in butter and then applied externally to sores (Watt and Breyer-Brandwijk, 1962). Infusions are also applied to haemorrhoids (Van Wyk *et al.*, 2005).

2.4.26.3 Phytochemistry/bioactivity

The roots of *C. baccifera* contain various secoiridoids, of which gentiopicrosid is the main component, together with small quantities of swertiamarine, chironoiside and others (Wolfender *et al.*, 1993). The bitter iridoids are known to stimulate appetite, but the compounds responsible for the healing properties appear to be unknown (Van Wyk *et al.*, 2005).

2.4.27 *Cissampelos capensis*

Cissampelos capensis L.f. (Menispermaceae); common names: dawidjiewortel (A). Plant parts used: rhizomes and roots.

2.4.27.1 Description

A perennial climber with twining stems and rounded, bright green leaves (Botha, 1980). The plant supports itself by twining around the stems of other plants. The flowers, borne in clusters are small, hairy and greenish and are followed by orange berries (Van Wyk *et al.*, 2005). It is widely distributed in the western parts of South Africa (Van Wyk *et al.*, 2005).

2.4.27.2 Medicinal uses

A well-know medicinal plant in the Western Cape which is traditionally used as a blood purifier for boils and syphilis, and also taken for bladder ailments, diarrhoea, colic and cholera (Watt and Breyer-Brandwijk, 1962). The Xhosa apply a paste of the leaves to wounds and sores (Watt and Breyer-Brandwijk, 1962). It is traditionally taken as a brandy tincture, as an infusion or decoction with *Pentzia incana* (Thunb.) Kuntze and *P. globosa* Less. and externally applied as a poultice (Watt and Breyer-Brandwijk, 1962). Fresh or dry

rhizomes are chewed or taken directly as an infusion or tincture for diabetes (Van Wyk and Gericke, 2000).

2.4.27.3 Phytochemistry/bioactivity

A large number of biologically active alkaloids of the bisbenzyltetrahydroisoquinoline type have been isolated from several *Cissampelos* species, of which cissampareine is a typical example (Van Wyk *et al.*, 2005). According to Watt and Breyer-Brandwijk (1962) *Cissampelos* species contains the alkaloid cissampeline. Sedative, antispasmodic and antitumour properties have been ascribed to Menispermaceae alkaloids (Anonymous, 1996; Bruneton, 1995).

2.4.28 *Harpagophytum procumbens*

Harpagophytum procumbens (Burch.) DC. ex. Meisn. (Pedaliaceae); common names: devil's claw, grapple plant (E); duiwelsklou (A); ghamaghoe (K). Plant parts used: secondary roots.

2.4.28.1 Description

A perennial plant with creeping, annual stems protruding from a fleshy corm. The leaves are blue-green on top and silver-grey underneath. The tubular flowers are a deep purple to pink with a yellow centre. The characteristic fruit has numerous "tentacles" with sharp, hooked thorns as well as two straight thorns on the upper surface (Van Rooyen, 2001). It occurs in sandy soils in the northwestern parts of southern Africa as well as the dune veld of the Kgalagadi Transfrontier Park (Van Rooyen, 2001).

2.4.28.2 Medicinal uses

H. procumbens is an important medicinal plant, the corms and roots are used for ailments of the gallbladder and kidneys, for diabetes, arteriosclerosis, osteoarthritis, rheumatism, ulcers, high blood pressure and fever (Van Wyk and Gericke, 2000; Van Rooyen, 2001). Dried root infusions are taken as a cure for digestive disorders and as a tonic (Van Wyk *et al.*, 2005). The fresh tuber is made into an ointment and applied to sores,

ulcers, boils and other skin lesions (Watt and Breyer-Brandwijk, 1962). An infusion of 1.5 g of powdered material in a cup of boiling water and strained, can be taken daily. Standardised extracts are available in the form of capsules, tablets, tinctures and ointments (Van Wyk *et al.*, 2005).

2.4.28.3 Phytochemistry/bioactivity

According to Van Wyk *et al.* (2005) the roots are rich in sugars but also contain phytosterols, triterpenoids and flavonoids. The active ingredients in the roots are considered to be a cinnamic acid ester, harpagoside, harpagide (possibly a degradation product of harpagoside) and procumbide (Czygan and Krüger, 1977; Pourrat *et al.*, 1986; Anonymous, 1996). According to Bruneton (1995), animal studies indicated slight analgesic and anti-arthritic effects. In Germany it is used in supportive therapy for degenerative disorders of the locomotor system, for lack of appetite and dyspeptic problems (Van Wyk *et al.*, 2005). A recent clinical study indicated effectiveness in the treatment of acute low backache (Chrubasik *et al.*, 1996).

2.4.29 Hoodia currorii

Hoodia currorii (Hook.) Decne. (Asclepiadaceae); common names: Ghaap; !khobab (K). Plant parts used: fleshy stem.

2.4.29.1 Description

These are leafless succulent plants with thick fleshy erect stems with rows of small thorns. The disc-like, flesh-coloured flowers smell strongly of decaying meat, attracting flies and blowflies for pollination (Van Wyk and Gericke, 2000). It occurs in the dry north-western parts of southern Africa.

2.4.29.2 Medicinal uses

Hoodia currorii is eaten as a food, used as an appetite-suppressant, and is used to treat indigestion, hypertension, diabetes and stomachache (Van Wyk and Gericke, 2000).

According to Von Koenen (2001) the plant is known as a diabetes remedy to the Damara people of Namibia.

2.4.29.3 Phytochemistry/ bioactivity

Hoodia species contains a pregnane glycosides P57 that suppress hunger (Van Wyk and Wink, 2004).

2.4.30 *Nymphaea nouchali*

Nymphaea nouchali Burm.f. var. *caerulea* (Savigny) Verdc. (Nymphaeaceae); common names: egyptian blue lily; sacred blue lily; blue water lily (E); blouwaterlelie, kaaimanblom (A); iZubu (Z). Plant parts used: seeds.

2.4.30.1 Description

This is a perennial hydrophyte with tuberous rhizomes anchored in pond mud by spreading roots. It does not have a true stem but the leaves are born on long leaf stalks that arise directly from the rhizome. The leaves are large, flat, and oval with smooth margins and a deep sinus where the petiole is attached. The showy, blue, bisexual flowers appear above the water at the tip of a sturdy stalk from September until April. Colour variations may occur varying from white to mauve. Numerous blue-tipped bright yellow stamens occupy the centre of the flower. In Africa this species occurs from tropical to southern Africa where it is common. In South Africa it is found in waterbodies in the Highveld, Lowveld as well as in KwaZulu-Natal (Viljoen and Notten, 2002).

2.4.30.2 Medicinal uses

The seeds of *Nymphaea nouchali* are used as a remedy for diabetes. An infusion of the root and stem is emollient and diuretic and is used in treating blenorrhagia and infections of the urinary passages. A decoction of the flower is said to be a narcotic as well as an aphrodisiac. The flowers have been used as a remedy for dysuria and for coughs (Watt and Breyer-Brandwijk, 1962).

2.4.30.3 Phytochemistry/bioactivity

N. nouchali contains the alkaloids nuciferine and apomorphine. Recent studies have also shown it to have euphoric properties (Perry *et al.*, 2002).

2.4.31 *Trigonella foenumgraecum*

Trigonella foenumgraecum L. (Fabaceae); common names: fenugreek; fenugrec (France); fieno Greco (Italian); alholva, feno-greco (Sp); helba (Arabic); methi (Indian). Plant parts used: seeds.

2.4.31.1 Description

A highly aromatic, erect, annual herb with trifoliolate oblong-lanceolate leaflets. Yellowish flowers are borne in the leaf axil. The fruit is a long, narrow, sickle-like pod containing the brownish oblong seeds that are divided by a furrow into two unequal lobes (Suttie, 2007). Fenugreek is originally from the Mediterranean regions, northeastern Africa and western Asia (Van Wyk and Wink, 2004), but currently cultivated in India and neighbouring countries as well as in France, Turkey, and China. Fenugreek grows on a wide range of well-drained soils (Suttie, 2007).

2.4.31.2 Medicinal uses

Fenugreek is an important medicinal plant and is used for the treatment of abdominal colic, bronchitis, coughs, sprains, diabetes, asthma, emphysema, gastrointestinal troubles, constipation, fever, sterility, a treatment after child birth, and also as a digestive, an abortive, a purgative, a galactagogue, an emmenagogue, a stomachic, reconstituent, sedative for palpitations, icterus, an anthelmintic and an aphrodisiac (Suttie, 2007). A herbal mixture consisting of 5 g of *Tylophora hirsuta* Wight. leaves, 25 g of *T. foenumgraecum* seeds and 50 g of the aerial parts of *Fumaria indica* in water is being used for diabetes (Mushtaq *et al.*, 2007).

2.4.31.3 Phytochemistry/bioactivity

The seeds are rich in mucilage, mainly galactomannans, lipids, proteins, and protease inhibitors (Van Wyk and Wink, 2004). Steroidal saponin and the aglycone diosgenin and its epimer yamogenin are found in the seed oil of fenugreek whereas the alkaloid trigonelline

was extracted from the seeds. Small amounts of an alkaloid, trigonelline, and a steroidal peptide, foenugraecin, may contribute to the medicinal properties. Fenugreek also contains the furostanol glycosides trigofenosides A/G (Van Wyk and Wink, 2004; Suttie, 2007). The saponins could be responsible for the observed antidiabetic, lipid and cholesterol lowering activities (Van Wyk and Wink, 2004).

2.4.32 *Vernonia oligocephala*

Vernonia oligocephala (DC.) Sch.Bip. ex Walp. (Asteraceae); common names: groenamara, bitterbossie (A); mofolotsane (SS); sefifatse (Ts); ihlambihloshane (Z). Plant parts used: aerial parts.

2.4.32.1 Description

It is an erect, perennial, herbaceous plant up to 1 m tall. The stems develop from a woody rootstock. The elliptic leaves are pale green above and silver below due to the presence of a velvet hair cover. The dark pink flowerheads are grouped together on the branch tips (Van Wyk and Malan, 1988). *V. oligocephala* is widespread throughout the grassland regions of South Africa (Van Wyk and Malan, 1988; Van Wyk *et al.*, 2005).

2.4.32.2 Medicinal uses

Infusions are taken as stomach bitters to treat abdominal pains and colic. It is also used for the treatment of rheumatism, dysentery and diabetes (Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings *et al.*, 1996). Infusions are made of the leaves (Van Wyk *et al.*, 2005).

2.4.32.3 Phytochemistry/bioactivity

Various sesquiterpenoid lactones have been isolated from *Vernonia* species (Anonymous, 1996), including germacranolides and glaucolides for example glaucolide A (Bohlman *et al.*, 1984).

2.5 Conclusions

The trade in traditional medicines forms part of a multi-million rand economy in southern Africa (Cunningham, 1997), stimulated by high population growth, rapid urbanization, unemployment, and a high cultural value of traditional medicines (Dold and Cocks, 2002). The trade is now greater than at any time in the past and is certainly the most complex resource managing issue facing conservation agencies, health care professionals and resource users in South Africa (Dold and Cocks, 2002).

The popularity of herbal medicines has led to increasing concerns over their safety, quality and efficacy. In many countries the herbal medicine market is poorly regulated and products are neither registered nor controlled. There is a lack of detailed documentation on the use of medicinal plants in South Africa. The need to document traditional knowledge is a priority because the rapid pace of urbanization and aculturation in this country could easily lead to the permanent loss of this knowledge (Van Wyk *et al.*, 2005).

A study was conducted to look at mortality from traditional medicine of patients admitted at Ga-Rankua Hospital, South Africa. The results of this study have reinforced the concerns of the Medicines Control Council about the safety of some traditional medicines. Some medicinal plants species used by traditional healers in South Africa have shown a significant degree of toxicity, which obviously outweighs their benefits (South African Traditional Medicine Research Unit, 2005).

In the quest for discovering new hypoglycaemic substances it is necessary to scientifically validate the claimed medicinal properties of traditional medicines. This inventory will assist researchers in the selection of plant species to evaluate for their hypoglycaemic activities. This study also gives an indication of the toxicity of some of the plant extracts where this information was available. The method of preparation and administration of medicines used by traditional healers is the starting point to design experimental protocols aimed at finding scientific evidence of efficacy and toxicity. The ability to produce safe, standardized medicinal plant products for further clinical evaluation is a major stumbling block in most countries wishing to enhance the quality of their traditional medicines (South African Traditional Medicine Research Unit, 2005).

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The hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes are discussed in this chapter.

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Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes

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3.1 Abstract

Aim: To validate selected plant species for hypoglycaemic activity.

Materials and methods: Four plant species were investigated for hypoglycaemic activity by evaluating the inhibiting effects on carbohydrate-hydrolysing enzymes; alpha-glucosidase and alpha-amylase. Acetone plant extracts were screened against C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells by measuring the glucose uptake. Cytotoxicity was conducted in preadipocytes and hepatocytes cell lines.

Results: Extract of *Euclea undulata* rootbark exhibited the highest activity, displaying a glucose uptake of 162.2% by Chang liver cells at 50 µg/ml. The fifty percent inhibitory concentration of the acetone extract of *E. undulata* was found to be 49.95 µg/ml and 2.8 µg/ml for alpha-glucosidase and alpha-amylase enzymes respectively. No cytotoxicity was recorded for *Euclea undulata*, while *Schkuhria pinnata* and *Elaeodendron transvaalense* exhibited cytotoxicity at 12.5 µg/ml. Alpha-glucosidase and alpha-amylase assays showed inhibitory activity on the enzymes for three plant extracts.

Conclusion: *E. undulata*, *S. pinnata* and *E. transvaalense* showed *in vitro* hypoglycaemic activity. *S. pinnata* and *E. transvaalense* indicated cytotoxicity on 3T3-L1 preadipocytes and Chang liver cells. *E. undulata*, *P. divaricata* and *E. transvaalense* inhibited alpha-glucosidase and alpha-amylase enzymes.

Ethnopharmacological relevance: The screening of plant extracts scientifically validated traditional use of *E. undulata* for treatment of diabetes. Cytotoxicity results revealed that the acetone extracts of *S. pinnata* and *E. transvaalense* are toxic and raised concern for chronic use.

Authors Keywords: Alpha-amylase; alpha-glucosidase; hypoglycaemic activity; *Euclea undulata*.

3.2. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and glucose intolerance, either due to insulin deficiency or to impaired effectiveness of insulin's action or to a combination of both. It is regarded as a non-curable but controllable disease. In Africa, diabetes mellitus is no longer a rare disease and recent investigations of non-communicable diseases indicated an increase in prevalence from 1% to 20% of the population. In South Africa the prevalence is between 4% and 6%. The global prevalence was estimated at 2.8% in 2000 (171 million people) and it is projected that 4.8% (366 million people) will be affected by 2030 if no action is taken (Wild *et al.*, 2004). The progressive nature of the disease necessitates constant reassessment of glycaemic control in people with diabetes and appropriate adjustment of therapeutic regimens. The burden of the disease is high not only because life-long treatment is necessary but also due to the prohibitive cost and unavailability of treatment in rural areas. The rate of limb amputations varies from 1.4% to 6.7% of diabetic patients, while foot complications and the annual mortality linked to diabetes worldwide is estimated at more than one million (World Health Organization, 2005). If glycaemic control is no longer obtained with a single agent, the addition of a second or third drug is usually more effective than switching to another single agent. Most patients with type II diabetes will ultimately require multiple anti-diabetic agents to maintain adequate glycaemic control (Gerich, 2001). The 2007 World Health Organization Regional office for Africa report stated that one of its strategy aims was to support research in community interventions, including traditional medicine. It is essential for the above reasons that the search for new anti-diabetic agents continues.

The most common problem encountered in the detection of pharmacological activity in plant extracts is that even extracts from single plants are mixtures of several compounds, and these can vary in concentration or composition depending on ecological changes (Farnsworth, 1993). Traditional remedies seldom comprise a single plant extract, and in many cases the therapeutic benefits are attributed to the consumption of plant mixtures in which whole plants or plant parts are prepared and consumed in combination (Etken, 1986). This complicates the pharmacological investigation of the preparation, because it has to be

determined which of the many constituents of a single plant is the active one. *In vitro* screening methods have a further inherent problem in that some compounds showing good activity in an *in vitro* assay may be metabolized *in vivo* into inactive metabolites. Conversely, some extracts show only *in vivo* activity due to the metabolism of inactive compounds into active compounds (Farnsworth, 1993). It is clear from the above that screening the activity of plant extracts may vary from batch to batch. However, the continued large scale use of plants in traditional medicines in developing countries, such as South Africa, necessitates the validation of the use of these plants for various diseases as being effective and safe.

The hypoglycaemic activity of four plant species traditionally used for the treatment of diabetes by South African traditional healers and herbalists was investigated. Species included the small herbaceous weed, *Schkuhria pinnata* (Lam.) Cabrera (Asteraceae). A decoction is prepared from the whole plant and used by traditional healers for the treatment of diabetes in the Ga-Rankuwa area, Gauteng, South Africa (Matibe, pers. comm.) (Deuschländer *et al.* 2009) *Pteronia divaricata* (P.J. Bergius) Less. (Asteraceae), is a twiggy, dense shrublet which grows up to 1 m tall. A tea is brewed from this plant and used in the treatment of diabetes by a traditional healer in the Clanwilliam district, Western Cape, (Maltz, pers. comm.) (Deuschländer *et al.* 2009). Both *Euclea undulata* var. *myrtina* Thunb. (Ebenaceae), a dense, erect, evergreen dioecious shrub or small tree and *Elaeodendron transvaalense* (Burt Davy) R.H. Archer (Celastraceae), a shrub or small, multi-branched tree from 5 to 10 m tall, are used in the Venda region, Limpopo Province, by local traditional healers and herbalists for the treatment of diabetes (Tshikalange, pers. comm.) (Deuschländer *et al.* 2009). A tea is brewed from the rootbark of *E. undulata* var. *myrtina* and from the stembark of *E. transvaalense* for this purpose.

3.3. Materials and Methods

3.3.1 Plant material

Plant material was obtained from traditional healers as well as collected in the veld at various localities. *S. pinnata* (Deuschländer 112982) was obtained from the

Medical University of South Africa at Ga-Rankuwa, Gauteng, and additional material was collected from the University of Pretoria's experimental farm. *P. divaricata* was obtained from a traditional healer in the Clanwilliam district, Western Cape. *E. undulata* (Deutschländer 95254) was collected in the Lydenberg district, Mpumalanga whereas *E. transvaalense* was collected in the Venda region, Limpopo, (Tshikalange 092524). Plant material was authenticated by Ms M. Nel and Prof. A.E. van Wyk at the H.G.W.J. Schweickert Herbarium, University of Pretoria where voucher specimens are being kept.

Entire *S. pinnata* and *P. divaricata* plants were used for the preparations of the crude extracts. Acetone and ethanol extracts were prepared for *S. pinnata* whereas only acetone extracts were prepared for *P. divaricata* and the rootbark and stembark of *E. undulata* and *E. transvaalense* respectively. An ethanol extract was also prepared for *S. pinnata* to compare the hypoglycaemic activity of ethanol and acetone extracts. Results obtained from ethanol and acetone extracts were more or less similar but the toxicity for the ethanol extracts was higher, hence acetone extract was chosen for other investigations. Acetone / ethanol were originally chosen as solvents as their polarities are close to that of water.

3.3.2. Preparation of plant extracts

Initially the air-dried plant material was ground by using a mill and weighed (*S. pinnata* 70g; *E. undulata* 28g; *P. divaricata* 24g and *E. transvaalense* 94g). Ground plant material was extracted with cold acetone except in the case of *S. pinnata*, where a separate ethanol extract was also prepared. The volume of solvent varied from 300 ml – 500 ml depending on the quantity of ground plant material (24 g-94 g). This procedure was repeated three times to ensure that all possible compounds would be extracted. The filtered plant extracts of the three repetitions were combined and dried by making use of a Rotavapor (Büchi R-114) and liquid nitrogen. The percentage yield obtained for the various plant extracts were as follow: *Schkuhria pinnata* (1.92%); *E. undulata* (3.05%); *P. divaricata* (4.98%) and *E. transvaalense* (0.91%).

3.3.3 *In vitro* anti-diabetic and toxicity screening

The *in vitro* anti-diabetic and toxicity screening were done by using a method with slight modifications as described by Van De Venter *et al.* (2008). This method measures glucose utilization and can be used for long-term exposure of the cells to the sample.

Plant extracts prepared (reconstituted in undiluted DMSO, vortexed and left for 15 minutes, before further dilution with the respective growth medium) were tested on three cell lines namely: Murine C2C12 myoblasts, Chang liver cells and 3T3-L1 preadipocytes (Highveld Biological, South Africa). The final DMSO concentration never exceeded 0.25% and a vehicle control was always included in each experiment. At this concentration of DMSO, no effects were seen on cell viability. Hepatic, preadipocytes and myocytes were used because hepatic and, preadipocytes and muscle cells have different glucose transporters, and these react differently to insulin stimulation and have different roles in carbohydrate metabolism. This method has the potential to detect not only alterations in glucose uptake but any changes that might occur in the metabolic pathways where glucose plays a role.

3.3.4. Routine maintenance of cell cultures

All cell cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂. The Chang liver and C2C12 cell lines were fed fresh growth medium every two to three days, consisting of RPMI1640 (Highveld Biological, South Africa) medium supplemented with 10% fetal bovine serum. The 3T3-L1 cells were cultured in DMEM (1.5g/l NaHCO₃) (Highveld Biological, South Africa) with 10% bovine serum.

3.3.4.1 Glucose uptake experimental procedures on C2C12 myocytes

Cells in monolayer culture (at less than 70% confluence) were dislodged through brief exposure to 0.25% trypsin in PBSA, counted and suspended (25 000 cells/ml) in growth medium (RPMI1640 with 10% fetal bovine serum) and the 200 µl/well seeded into new 96-well plates. The seeded plates were incubated at 37°C for 3 to 4 days without changing the medium.

To measure glucose uptake, insulin (1 μM) was used as positive control whereas 50 $\mu\text{g}/\text{ml}$ plant extract (acetone for all four plants, as well as ethanol for *S. pinnata*) firstly in incubation medium and then the Glucinet reagent kit (Adcock Ingram) were used to execute the assays.

All medium were aspirated from the cells and then 50 μl of the incubation medium/insulin/plant extract was added into the microtiter wells and incubated for one hour. Thereafter 20 μl was transferred from each well to a clean well plate and 200 μl Glucinet reagent was added per well. The plates were incubated at 37°C for 15 minutes and the absorbance was read at 492 nm using a microplate reader. Due to the short exposure of the C2C12 myocytes to the plant extracts no toxicity assay were done on this particular cell line.

3.3.4.2 Glucose uptake experimental procedures on Chang liver cells and 3T3-L1 preadipocytes

Chang liver cells and 3T3-L1 preadipocytes (at less than 90% confluence) were dislodged through brief exposure to 0.25% trypsin in PBSA, counted, suspended (30 000 cells/ml) in growth medium (RPMI1640 with 10% fetal bovine serum) and then 200 μl /well seeded into new 96-well plates. The seeded plates were incubated at 37°C for five days without changing the medium. On day three the plant extracts or positive control was administered to the relevant vials according to the plate-well layout by adding 10 μl extract or positive control to each well to a final concentration of 12.5 $\mu\text{g}/\text{ml}$ extract or 1 μM metformin (for Chang liver cells). Incubation was continued until day five when the glucose uptake experiment was done. The positive control for 3T3-L1 preadipocytes was 1 μM insulin but this was only added on day five during the glucose uptake experiment in order to avoid the development of insulin resistance. Fifty $\mu\text{g}/\text{ml}$ crude plant extract in the incubation medium (RPMI1640 with 8 mM glucose, 0.1% BSA) and Glucinet reagent (Sigma) was used to execute the assays as was done for C2C12 cells, except the incubation period was 1.5 hours for 3T3-L1 and 3 hours for Chang liver cells. Toxicity assays were done by adding MTT (0.5 mg/ml in RPMI1640:10% fbs) to the wells of the last three rows of each plate. MTT is a yellow water soluble tetrazolium dye that is reduced by living

cells, but not dead cells, to a purple formazan product that is insoluble in aqueous solutions (Mosmann, 1983). Toxicity results were compared using Student's *t*-test (Two-sample assuming equal variances P (T<=t) two tail) with $p < 0.05$ considered as significant.

3.3.4.3. Dose response assay

The best hypoglycaemic effect was obtained from the acetone extract of *E. undulata*, a dose response assay was conducted on Chang liver cells for this extract. The concentration used ranged from 16 $\mu\text{g/ml}$ to 250 $\mu\text{g/ml}$.

3.4 Alpha-glucosidase inhibiting activity

Alpha-glucosidase is an enzyme produced by the villi lining the small intestine of mammals which is responsible for the hydrolysis of disaccharides to monosaccharide that can then be absorbed and consequently elevate blood glucose levels. Inhibition of intestinal α -glucosidase has been used successfully to treat patients with both type I and II diabetes mellitus (Collins *et al.*, 1997; Hiroyuki, *et al.*, 2001).

The alpha-glucosidase inhibiting activity of the four acetone plant extracts was tested following the method described by Collins *et al.* (1997). This microplate assay offers convenience, speed and reproducibility. The alpha-glucosidase inhibitory activity was determined by measuring the release of p-nitrophenol from p-nitrophenyl- α -D-glucopyranose. The released p-nitrophenol yields a yellow colour when the stopping reagent, glycine (pH10), is added.

Two milligrams of each of the plant extracts were dissolved in 1 ml 100% DMSO to prepare a stock solution and 1mg acarbose was dissolved in 1 ml buffer to be used as the positive control (Subramanian *et al.*, 2008). The plant extract stock solutions were subsequently diluted with buffer (1,2-Morpholinnoethane sulfonic acid monohydrate–NaOH) (Mes-NaOH) (pH 6.5) (Fluka 69892) to obtain a final concentrations ranging from 0.02 to 200.0 $\mu\text{g/ml}$. The substrate consisting of 1 g p-nitrophenyl- α -D-glucopyranose (Sigma-Aldrich, N1377-1G) was dissolved in 5 ml buffer and incubated at 37° C for 15 minutes. The enzyme (α -glucosidase type 1 from Bakers Yeast) (Sigma 63412) was prepared and used at the highest concentration (0.58 $\mu\text{g}/\mu\text{l}$). The plant extract, positive

control, enzyme, buffer and substrate were placed in the 96-well microtiter plate to a volume of 200 μ l. After an incubation period of 15 minutes at 37° C in a Labcon incubator the reaction was stopped by adding 60 μ l glycine (pH10). The absorbance was read at 412 nm in a microtitre plate reader.

3.5 *Alpha-amylase inhibiting activity*

Alpha-amylase is a glycoside hydrolase enzyme that breaks down long-chain carbohydrates by acting on the α -1,4-glycosidic bonds, yielding maltotriose and maltose molecules from amylose, or maltose and glucose from amylopectin. Two types of amylase are found in the human body, one in the saliva that breaks down starch into maltose and dextrin, better known as ptyalin and the other pancreatic α -amylase that cleaves the α -1,4-glycosidic linkages of amylose to yield dextrin, maltose or maltotriose.

The alpha-amylase inhibiting activity of the four plant extracts were tested based on the methods described by Park and Johnson (1949), Bernfeld (1955) and Slaughter *et al.* (2001). According to this method the reduction of ferricyanide ions in alkaline solutions followed by the formation of Prussian blue (ferric ferrocyanide) is measured quantitatively as the basis for the estimation of glucose levels. The alpha-amylase assay consists of two steps namely the enzyme and the Prussian blue assays.

3.5.1. *Enzyme assay*

The enzyme assay was executed in 32, 20 ml Pyrex test tubes. Distilled water was added to the test tubes (blank 2.5×10^3 μ l, negative control 1.25×10^3 μ l, assay 1×10^3 μ l and the colour test 2.25×10^2 μ l). Plant extracts and the positive control (acarbose) were added to the test tubes for the assay as well as for the colour test (250 μ l) at four different concentrations namely 1.25×10^3 , 1.0×10^3 , 7.5×10^2 and 5.0×10^2 μ g/ml (Subramanian *et al.*, 2008). Alpha-amylase type VI-B from porcine pancreas (Sigma A; 3176-1MU) was prepared fresh by dissolving 20 μ g porcine pancreatic enzyme in 30 ml ice cold distilled water, sonicated for 15 minutes and placed on ice. The enzyme (1.25×10^3 μ l) was added to the test tubes containing the negative and positive controls as well as the assays and incubated at 25° C for 5 minutes in a warm water bath. Potato starch (Sigma EC232-686-4) (0.5%) was prepared by dissolving 60 μ g in 120×10^3 μ l sodium phosphate buffer (pH 6.9).

Starch and buffer solution was heated for 20 minutes at a temperature of 60 – 70 °C. The prepared starch ($2.5 \times 10^3 \mu\text{l}$) was added to all the test tubes and placed in a warm water bath at 37°C for 3 minutes to activate the enzyme reaction.

3.5.2 Prussian blue assay

Thirty two Eppendorf tubes (2ml) were prepared each containing 300 μl sodium carbonate (Na_2CO_3) to which 300 μl sample from each of the porcine enzyme test tubes were added and centrifuged. A second set of 32 Eppendorf tubes (2ml) was prepared containing 950 μl of distilled water each to which 50 μl from the first set of Eppendorf tubes were added.

A second set of 32, 20 ml Pyrex test tubes were used and 500 μl of solution A (16 mM KCN, 0.19 M Na_2CO_3) and 500 μl of solution B (1.18 mM $\text{K}_3\text{Fe}(\text{CN})_6$) were added to each of these test tubes. A 500 μl of sample was taken from the second set of 32 Eppendorf tubes and added to the second set of test tubes containing solution A and solution B, these were vortexed, covered with foil and placed in boiling water for 15 minutes, removed and placed at room temperature for 15 minutes to cool down. Solution C ($2.5 \times 10^3 \mu\text{l}$) (3.11 mM $\text{NH}_4\text{Fe}(\text{SO}_4)_2$; 0.1 g sodium dodecyl sulphate in 1000 ml 0.05 N H_2SO_4) was added and left for 2 hours and 30 minutes in the dark for colour development. The absorbance was read at 690 nm on a Beckman Coulter DU 720 General Purpose UV/Vis spectrophotometer.

3.6. Results

3.6.1 *In Vitro*

The results obtained for *in vitro* assay on the C2C12 myocytes on the plant extracts (at 50 $\mu\text{g}/\text{ml}$), indicated that *E. undulata* showed some potential in lowering blood glucose levels (162.2%; 100% serves as base line). This was also observed for *S. pinnata* (ethanol) (107.5%; 100% serves as base line) (Figure. 3.1). Not much activity was observed when extracts of *S. pinnata* (acetone), *P. divaricata* or *E. transvaalense* were used (Figure. 3.1).

The toxicity of these plant extracts in C2C12 myocytes was not determined because of the short exposure time of the cells to the plant extracts (Van de Venter *et al.*, 2008).

The *in vitro* assay in 3T3-L1 preadipocytes indicated that *S. pinnata* (ethanol) (248.2%) and *Schkuhria pinnata* (acetone) extracts (179.6%) had some potential to lower blood glucose levels at a concentration of 50 µg/ml as did that of *E. transvaalense* (138.6%), and to a lesser extent *E. undulata* (126.0%) whereas *P. divaricata* showed no glucose uptake (Figure.3.1).

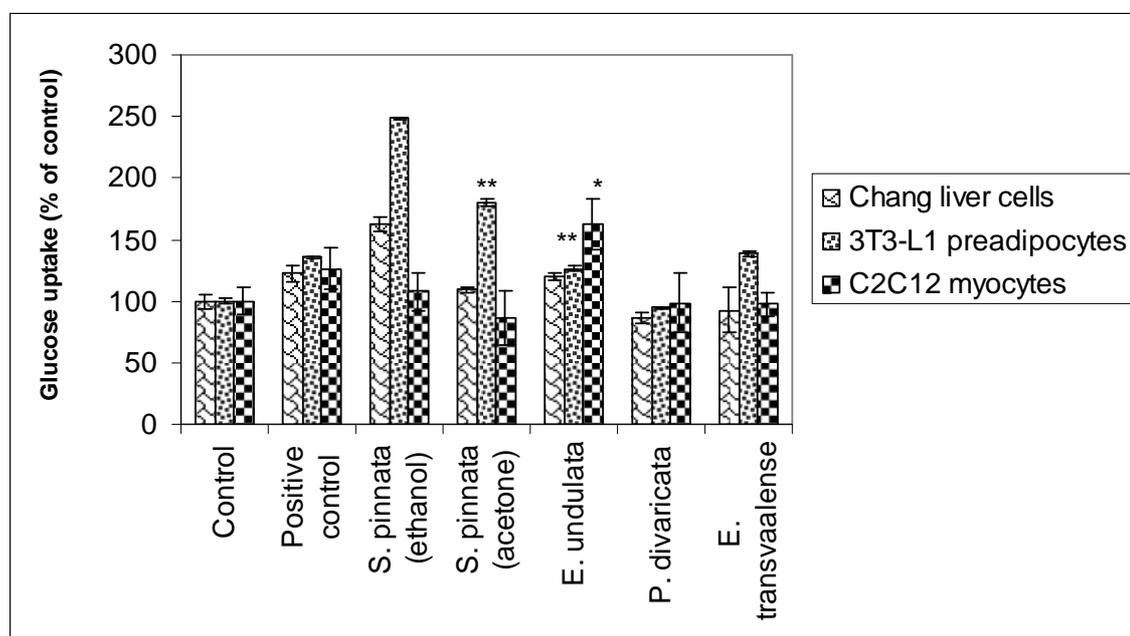


Figure 3.1. Glucose uptake (as % of control \pm standard error of mean, N = 8 for C2C12 and 10 for Chang liver and 3T3-L1) of the various plant extracts tested for their hypoglycaemic activity. (* P < 0.05; ** P < 0.005 compared to control using student's one and two-tailed t-test).

The toxicity assay revealed, however, that the results obtained with *S. pinnata* (ethanol) and (acetone) extracts were unreliable due to the toxicity of these extracts on 3T3-L1 preadipocytes (Figure.3.2). It should be noted that the toxic effect was induced by exposure of cells to an extract concentration of 12.5 µg/ml for 48 hours prior to measurement of glucose uptake in the presence of 50 µg/ml incubation medium for 1.5 hours. The *in vitro* assay in Chang liver cells indicated that *S. pinnata* (ethanol) extract (162.3%) and to a

lesser extent *E. undulata* extract (119.7%) had some potential at a concentration of 50 µg/ml. *P. divaricata* as well as *E. transvaalense* extracts showed no potential (Figure. 3.1). The toxicity assay revealed, however, that *E. transvaalense* and *S. pinnata* (ethanol) extracts were toxic to Chang liver cells (Figure.3.2).

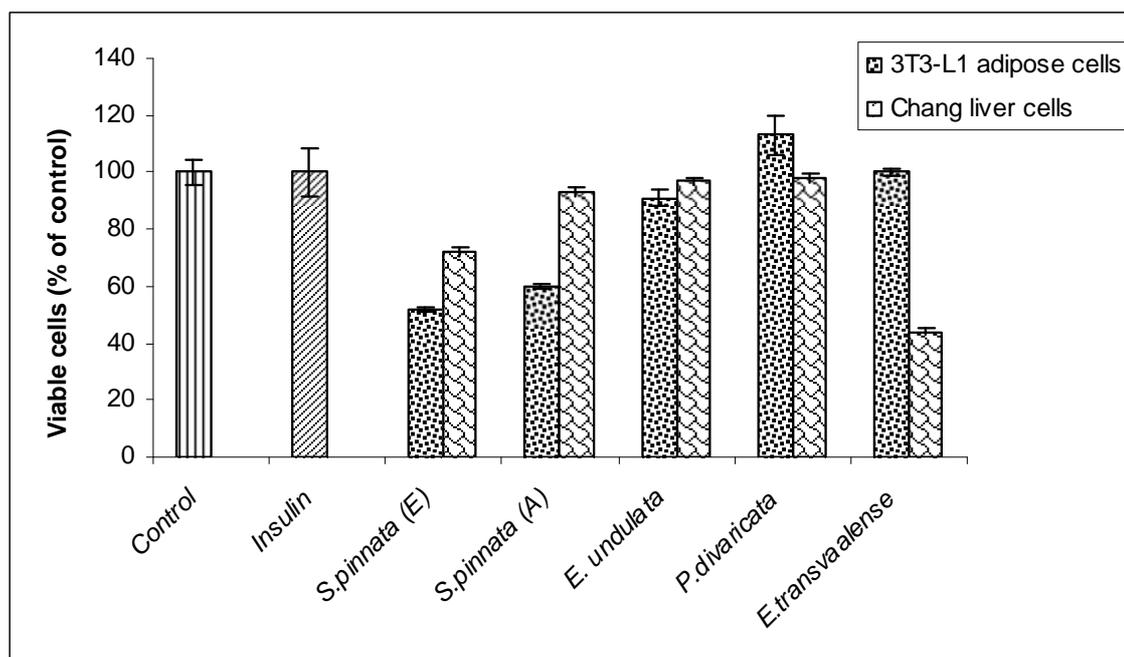


Figure 3.2. Toxicity (as % of control \pm standard error of mean, N = 6) of the various plant extracts tested for their hypoglycaemic activity.

The *in vitro* assay results indicated that four of the five plant extracts tested namely *S. pinnata* (ethanol), *S. pinnata* (acetone), *E. undulata* and to a lesser extent *E. transvaalense* showed positive results in increasing glucose uptake by 3T3-L1 preadipocytes, C2C12 myocytes and by Chang liver cells (Figure. 3.1). *P. divaricata* showed no ability in increasing glucose uptake.

The results obtained were interpreted by making use of the scoring system developed by Van de Venter *et al.*, (2008) to determine which plant extract to analyze further. According to this scoring system the potential anti-diabetic activity as well as the toxicity of the plant extract is taken into consideration to assist in the selection of the most active and least toxic plant extract (Table 3.1).

According to the results obtained from the different *in vitro* assays done on the various plant extracts *E. undulata* (score +3) was chosen for further analysis because, it was not toxic and it showed some hypoglycaemic activity in all three cell lines tested.

Table 3.1. Effect of plant extracts on glucose utilization and toxicity in Chang liver cells, 3T3-L1 preadipocytes and C2C12 myocytes using the scoring system of Van De Venter *et al.* (2008).

Species	Chang liver cells		3T3-L1		C2C12	Activity score (max +6)	Toxicity score (min -4)	Total score
	Active	Toxic	Active	Toxic	Active			
<i>S. pinnata (E)</i>	+2	-2	+2	-2	0	+4	-4	0
<i>S. pinnata (A)</i>	0	0	+2	-2	0	+4	-4	0
<i>E. undulata</i>	+1	0	+1	0	+1	+3	0	+3
<i>P. divaricata</i>	0	0	0	0	0	0	0	0
<i>E.transvaalense</i>	0	-2	+1	0	0	+1	-2	-1

Dose response assays were carried out *in vitro* on *E. undulata* extract to determine the hypoglycaemic effect on Chang liver cells at different concentrations. Figure 3.3 depicts the glucose uptake at different concentrations with the highest concentration being at 250 µg/ml and the lowest at 16 µg/ml. The highest glucose uptake was obtained at a concentration of 125 µg/ml (143.4%) and the lowest at 16 µg/ml (112.1%). The results obtained were statistically analyzed by making use of the Anova, One-way, Post Hoc, Tukey HSD test. According to the results, a value of $p < 0.05$ was considered significant, obtained at concentrations 125 µg/ml (0.042), 25 µg/ml (0.030), 0.50 µg/ml (0.00014) and 1µg/ml (0.00014).

3.6.2 MTT toxicity assay

The MTT toxicity assay revealed that *S. pinnata* (ethanol) ($p < 0.0001$) and (acetone) ($p < 0.0001$) extracts were toxic to 3T3-L1 preadipocytes. The same was found for the *E. transvaalense* ($p < 0.02$) extract. The *S. pinnata* ethanol ($p < 0.0001$) and the *E. transvaalense*

($p < 0.0001$) extracts were also toxic to Chang liver cells (Figure.3 2). The results were compared using the student's one and two tail t-test and P values obtained were < 0.0001 for some of the results. This can be explained by the large differences between control and test values in these treatments (all greater than 40%) and small variations between replicates.

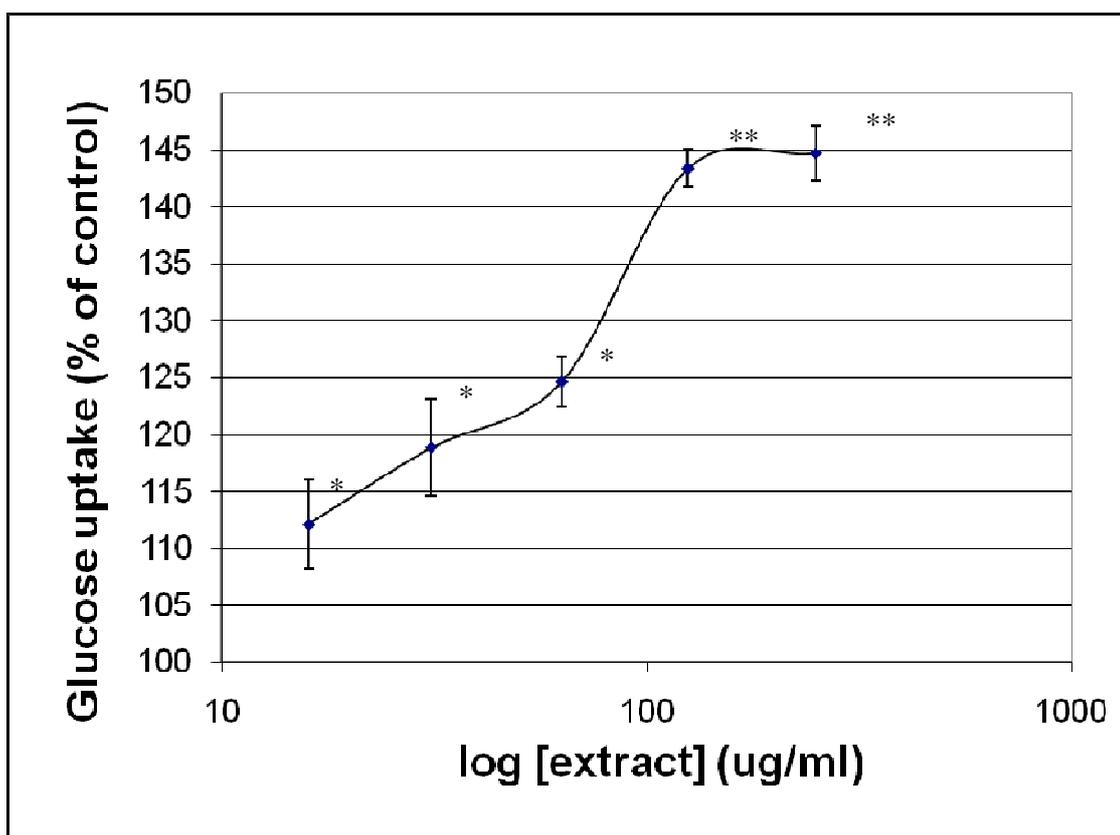


Figure 3.3. Dose response of *E. undulata* plant extract *in vitro* in Chang liver cells (as % of control \pm standard error of mean, N = 5). Cells were exposed to 12.5 μ g/ml extract for 48 hours before glucose utilization was measured in the presence of the extract concentrations as indicated. * $p < 0.05$; ** $p < 0.005$ compared to untreated control (using student's two-tailed t-test)

3.6.3 Alpha-glucosidase and alpha-amylase assay

The alpha-glucosidase and alpha-amylase 50% inhibitory concentrations (IC_{50}) of the plant extracts, as well as the positive control, acarbose, is depicted in Table 3.2. It is evident from the results that *P. divaricata* inhibited alpha-glucosidase the most, exhibiting an IC_{50} value

of 31.22 ± 0.35 $\mu\text{g/ml}$. It was evident from the results obtained with the alpha-amylase assays that acetone extracts of *E. undulata* and *E. transvaalense* inhibited alpha-amylase most with IC_{50} values of 2.80 ± 0.063 and 1.12 ± 0.079 $\mu\text{g/ml}$ respectively. IC_{50} value of 4.75 ± 3.18 and $22.92\mu\text{g/ml}$ was obtained for the positive control, acarbose, alpha-glucosidase and alpha-amylase respectively. Acetone extracts of *S. pinnata* showed no inhibition.

Table 3.2. Inhibitory effect (IC_{50}) of four plant extracts and positive control, acarbose for alpha-glucosidase and alpha-amylase.

Species	$\text{IC}_{50}^{\text{a}}$ ($\mu\text{g/ml}$) alpha-Glucosidase	$\text{IC}_{50}^{\text{a}}$ ($\mu\text{g/ml}$) alpha-Amylase
Acarbose	4.75 ± 3.18	22.92
<i>S pinnata</i> (A)	NI ^b	NI ^b
<i>E undulata</i>	49.95 ± 0.007	2.80 ± 0.063
<i>P. divaricata</i>	31.22 ± 0.154	36.30 ± 4.624
<i>E.transvaalense</i>	50.62 ± 0.351	1.12 ± 0.079

$\text{IC}_{50}^{\text{a}}$ = Fifty percent inhibitory concentration

NI^b = no inhibition

3.7 Discussion

The *in vitro* hypoglycaemic analysis revealed that the acetone extract of *S. pinnata*, *E. undulata* and *E. transvaalense* and ethanol extract of *S. pinnata* displayed hypoglycaemic activity in one or more of the various cell lines tested. While one acetone plant extract, *P. divaricata*, showed no hypoglycaemic activity in any of the cell lines tested. It was, however, determined that the high positive values obtained for *S. pinnata* could not be considered, as the toxicity assays revealed that the acetone and ethanol plant extracts are highly toxic to 3T3-L1 preadipocytes and the toxicity may be responsible for the high values obtained in the 3T3-L1 preadipocytes *in vitro* assays. Muthaura *et al.*,

(2007) noted a low cytotoxicity of methanol and water extracts of *S. pinnata* to Vero E6 cells but no toxicity was observed in *in vivo* mice tests. The acetone extract of *E. transvaalense* though displayed hypoglycaemic activity, but it was toxic to Chang liver cells. The only plant extract tested that displayed positive results in all three cell lines and no toxicity was *E. undulata*.

Some anti-diabetic drugs act through inhibition of digestion of complex carbohydrates in the gastrointestinal tract. To determine if some of the plant extracts could act at this level, they were tested to determine their inhibition of alpha-glucosidase and alpha-amylase. The results obtained for alpha-glucosidase and alpha-amylase indicated that the plant extracts of *E. undulata* (IC_{50} 49.95 ± 0.007 ; 2.80 ± 0.063), *E. transvaalense* (IC_{50} 50.62 ± 0.154 ; 1.12 ± 0.079) and *P. divaricata* (IC_{50} 31.22 ± 0.351 ; 36.30 ± 4.62) displayed alpha-glucosidase and alpha-amylase inhibition. *S. pinnata* displayed no alpha-glucosidase or alpha-amylase inhibiting activity. Of interest is the fact that *P. divaricata* did not display any *in vitro* hypoglycaemic activity but did show some alpha-glucosidase and alpha-amylase inhibiting action, indicating a possible different mechanism through which it could function as an anti-diabetic treatment.

Tshikalange (2007) isolated various triterpenoides such as lup-20(30)-ene-3,29-diol, (3 α)-(9Cl), lup-20(29)-ene-30-hydroxy-(9Cl), taraxastanonol, β -sitosterol and a phenolic derivative and depside namely ataric acid and atranorin from *E. transvaalense*. These substances were however not tested for their anti-diabetic activity, but all except β -sitosterol demonstrated alpha-glucosidase and alpha-amylase inhibition (Nkobole, 2009). Drewes and Mashimye (1993) isolated the phenolic compound, elaeocyanidin, as well as the gallotannins and ouratea proanthocyanidin A from *E. transvaalense* and it is likely that these compounds are responsible for the hypoglycaemic activity (Gruendel *et al.*, 2007; Gorelik *et al.*, 2008). In literature the use of *S. pinnata* as an anti-diabetic agent is numerous but no literature could be found on the chemical analysis and testing for its hypoglycaemic activity. Zdero *et al.* (1990) isolated some neryl geraniol derivatives, clerodane and three diterpenes from *P. divaricata*. The triterpenes betulin and lupeol were isolated from *E. natalensis* by Khan and Rwekika, (1992) and Weigenand *et al.*, (2004). According to Sudhahar *et al.* (2006) lupeol and its derivative normalized the lipid profile in

Wistar rats that were fed a high cholesterol diet. Ali *et al.*, (2006) also found that lupeol inhibited alpha-amylase enzyme. The positive results obtained in the present study could be attributed to the above mentioned isolated compounds.

Based on previous phytochemical studies and the results from this study, we conclude that *E. undulata* should be further investigated to identify the compounds responsible for its promising *in vitro* anti-diabetic activity.

3.8 Acknowledgements

The author would like to thank the Diabetes Research Group in the Department of Biochemistry and Microbiology at the Nelson Mandela Metropolitan University for their support and Prof Gretel van Rooyen for her assistance in the technical editing of the article. This research was financially supported by the National Research Foundation.

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CHAPTER 4

Chapter 4 deals with the phytochemical study conducted on the root bark of *Euclea undulata* Thunb. var. *myrtina* and the isolation of various compounds as well as their hypoglycaemic activity.

The isolation of a new triterpene and other compounds with hypoglycaemic activity, from *Euclea undulata* Thunb. var. *myrtina* (Ebenaceae) root bark

4.1 Abstract

Phytochemical studies of a crude acetone extract of the root bark of *E. undulata* var. *myrtina*. afforded a new triterpene (**1**), in addition to three known compounds betulin (**2**), lupeol (**3**) and epicatechin (**4**). The chemical structures were determined by spectroscopic means.

The hypoglycaemic activity of the four compounds isolated was determined by *in vitro* screening of glucose utilization by C2C12 myocytes at a concentration of 25 µg/ml or 50 µg/ml. The inhibition of carbohydrate-hydrolysing enzymes were also established at concentrations ranging from 0.02 to 200.00 µg/ml. The *in vitro* results on C2C12 myocytes indicated that compound **4** has the potential to lower blood glucose levels, whereas compound **1** has the ability to inhibit alpha-glucosidase at a concentration of 200.0 µg/ml.

4.2 Introduction

E. undulata Thunb. var. *myrtina* (Ebenaceae), a dense, erect, dioecious shrub or small tree was selected for the identification of bio-active principles after preliminary *in vitro* screenings were done for hypoglycaemic activity on an acetone extract of the root bark. This selection was based on the facts that the crude acetone extract of *E. undulata* root bark gave positive results (hypoglycaemic activity) in the *in vitro* assays done on C2C12 myocytes, 3T3-L1 preadipocytes and in Chang liver cells without displaying any toxicity and scored a +3 according to the scoring system developed by Van de Venter *et al.* (2008). The carbohydrate-hydrolysing enzymes alpha-amylase and alpha-glucosidase were also inhibited to some extent (Deuschländer *et al.*, 2009).

4.3. Materials and methods

4.3.1 Plant material

Plant material was collected at De Wagensdrift, Gauteng Province in August 2005. Voucher specimens (Deuschländer nr 95254) have been deposited at the H.G.W.J. Schweickert Herbarium, University of Pretoria and authenticated by Ms M. Nel.

4.3.2 Extraction of the plant material

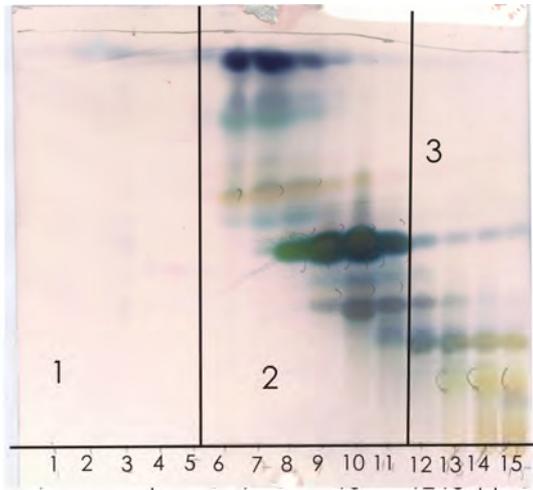
Plant material was air dried and the root bark stripped from the roots before it was ground. The ground root bark (215 g) was soaked in 0.5 l cold acetone for three days while on a shaker. After three days the extract was filtered and the residue extracted again with fresh cold acetone (3X). The plant extracts were combined and evaporated using a rotatory evaporator to yield 87 g (40 %) total extract.

4.3.3 Fractionation of the crude extract

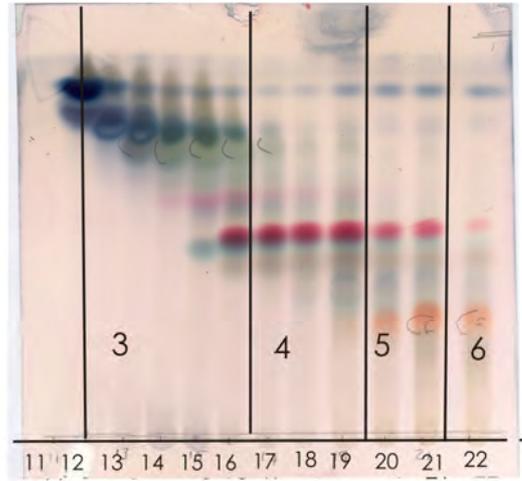
The crude acetone extract (35 g) was subjected to silica-gel column chromatography for the isolation of bioactive principles. The column was eluted with hexane: ethyl acetate mixtures of increasing polarity (0 – 100% ethyl acetate), washed with 100% methanol. Fractions containing the same compounds as determined by thin layer chromatography (TLC) were combined. Nine main fractions were obtained (Figure 4.1). All fractions except fractions 1 and 6 were not tested for glucose utilization using C2C12 myocytes as these fractions could not be dissolved in the solvent used for the bioassay. The bioactive fractions were further subjected to column chromatographic purification for the identification of bioactive principles. Fraction 2 was chromatographed over a silica column eluted with hexane: ethyl acetate mixtures of increasing polarity (0 – 100% ethyl acetate) and yielded (2500 mg; 7.14% yield) lupeol **3**. Fractions 3 and 4 were combined and chromatographed over a sephadex column using ethanol and yielded a new triterpene **1** (14.28 mg; 0.04% yield) and betulin **2** (20.01 mg; 0.06% yield). Fraction 8 was chromatographed over a sephadex column eluted with ethanol and yielded (12.02 mg; 0.03% yield) epicatechin **4** (Figure 4.2).

4.4 Determination of hypoglycaemic activity

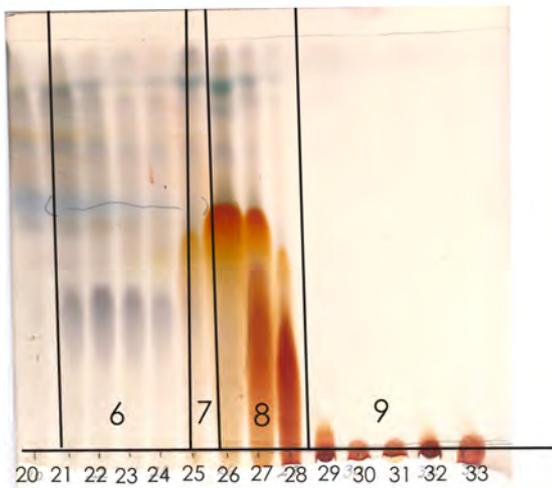
Seven of the nine main fractions obtained from the column chromatography were tested *in vitro* on C2C12 myocytes to measure glucose uptake at a concentration of 12,5 µg/ml and insulin (1 µM) in incubation medium was used as positive control. Glucinet reagent kit (Adcock Ingram) was used to execute the assays. According to the results obtained, fractions 2, 3 and 8 showed activity and subsequently submitted to chromatographic processes to isolate those compounds with probable hypoglycaemic activity.



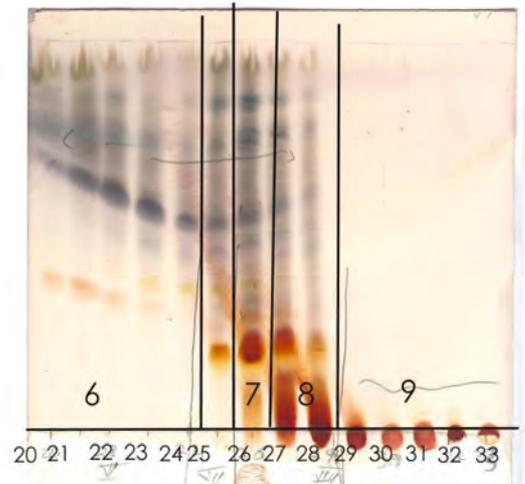
hexane: ethyl acetate (8:2)



hexane: ethyl acetate(1:1)



ethyl acetate : hexane (8:2)



methanol : chloroform (9:1)

Figure 4.1. Silica gel column fractionation of the acetone extract of *E. undulata*.

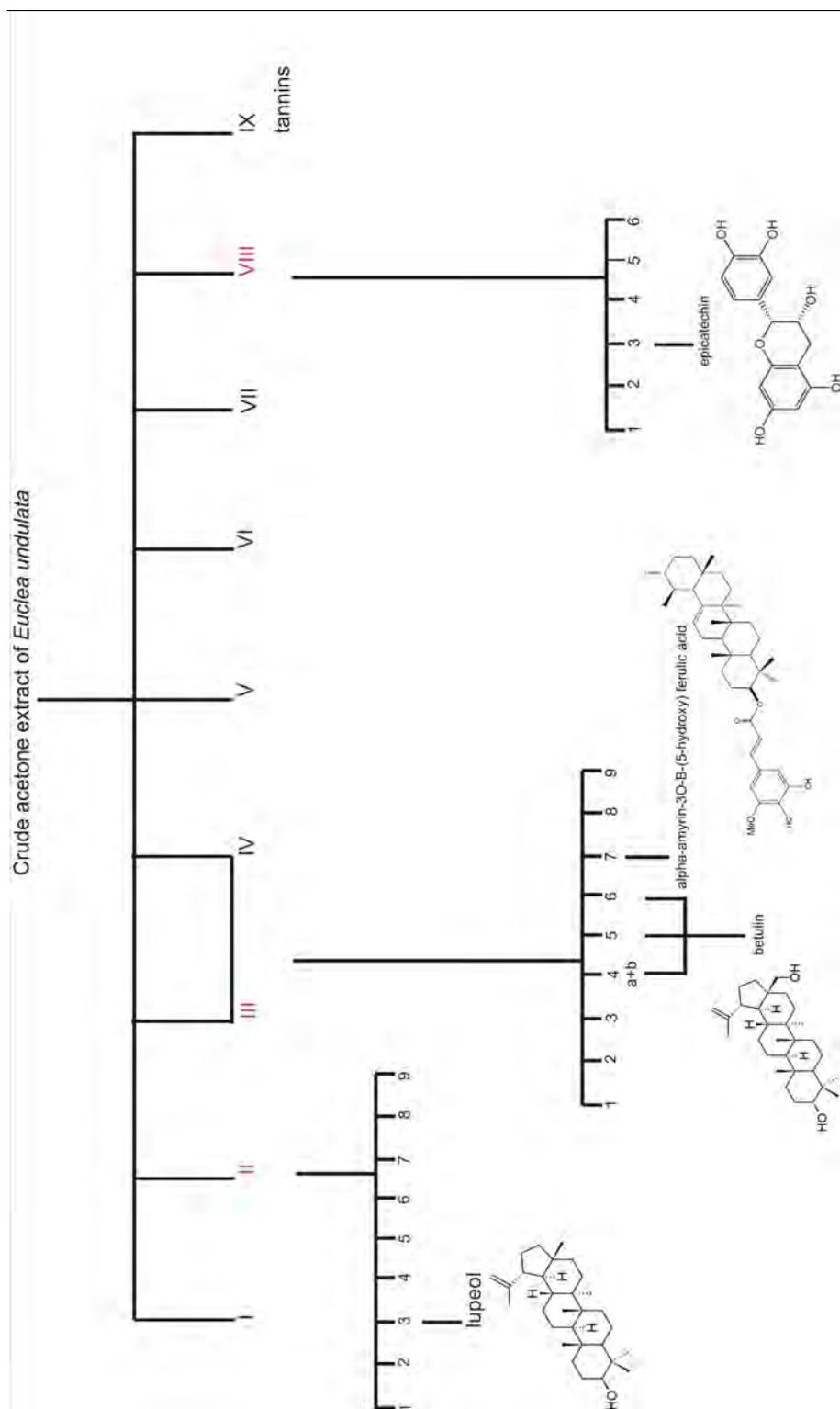


Figure 4.2. Dendrogram indicating the isolation of compounds from the crude acetone extract of *E. undulata*.

The hypoglycaemic activity of the isolated compounds **1** - **4** were also determined in C2C12 myocytes by applying the above mentioned assay. Concentrations used were 50 µg/ml for compounds **1** (81 µM), **2** (113 µM) and **3** (117 µM) and 25 µg/ml for compound **4** (86 µM), while insulin (1 µM) was used as positive control. Due to the lower molecular weight of epicatechin (290.3) compared to that of betulin (442.7), lupeol (426.7) and α -amyrin-3O- β -(5-hydroxy) ferulic acid (619.5), it was decided to test epicatechin at 25 µg/ml and the others at 50 µg/ml to yield more comparable molar concentrations ranging from 86 µM for epicatechin to 117 µM for lupeol.

The alpha-glucosidase inhibiting activity of the isolated compounds, **1** - **4**, was tested following the colorimetric micro-plate method as described by Collins *et al.* (1997). The alpha-glucosidase inhibitory activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl- α -D-glucopyranose. The released *p*-nitrophenol yields a yellow colour when the stopping reagent glycine (pH 10), is added. The various compounds (1 mg) were dissolved in 1 ml 100% DMSO to prepare a stock solution and 1mg acarbose was dissolved in 1 ml buffer (pH 6.5) (1,2- morpholinnoethane sulfonic acid monohydrate-NaOH) (Mes-NaOH) (Fluka 69892) to be used as a positive control (Subramanian *et al.*, 2008). Final concentration of acarbose used was 1 µM. The stock solutions of the different compounds were subsequently diluted with Mes-NaOH buffer to obtain final concentrations ranging from 0.02 to 200.0 µg/ml. The substrate consisting of 1 g *p*-nitrophenyl- α -D-glucopyranose (Sigma-Aldrich, N1377-1G) was dissolved in 5 ml buffer and incubated at 37° C for 15 minutes. The enzyme (α -glucosidase type 1 from Bakers Yeast) (Sigma 63412) was prepared and used at the highest concentration (0.58 µg/ml). The different compounds, positive control, enzyme, buffer and substrate were respectively placed in a 96-well microtiter plate well to a volume of 200 µl. After an incubation period of 15 minutes at 37° C in a Labcon incubator the reaction was stopped by adding 60 µl glycine (pH 10). The absorbance was read at 412 nM using a microtitre plate reader. The various methods were applied to the isolated compounds to establish if the different compounds exhibit different mechanisms to lower blood glucose levels. Due to the unavailability of sufficient amount of the purified compounds they could not be tested for alpha-amylase inhibitory activity.

unavailability of sufficient amount of the purified compounds they could not be tested for alpha-amylase inhibitory activity.

4.5. Results and discussion

4.5.1 *In vitro* assay results

Seven of the nine main fractions were investigated for hypoglycaemic activity *in vitro* on C2C12 myocytes by using a method developed by Van de Venter *et al.* (2008). This method measures glucose utilization and can be used with long-term exposure of cells to the sample. It was found that fractions II, III and VIII were active (Figures 4.1 and 4.,2).

The results obtained from the *in vitro* assay on C2C12 myocytes indicated that fractions II (44.8%) (100% used as base line), III (50.6%) and VIII (82.8%) showed potential to lower blood glucose levels (Figure 4.3) Consequently fractions II, III and VIII were subjected to the isolation processes using different chromatographic techniques to isolate the pure, active compounds. The purification process of the above mentioned fractions resulted in the isolation of compounds **1 - 4**.

The *in vitro* assay on C2C12 myocytes of the different compounds revealed that **4** was active (266.3%) in lowering blood glucose levels at a concentration of 25 µg/ml and **2** was active to a lesser extent (121.4%) at a concentration of 50 µg/ml (100% used as base line) (Figure 4.4). In literature no evidence could be found of compounds 1- 4 being tested for hypoglycaemic activity on C2C12 myocytes.

4.5.2 *Alpha-glucosidase* assay

According to the results obtained from the alpha–glucosidase assays on the different compounds isolated from the root bark of *E. undulata* an inhibition of 68%, 39% and 40% was found on exposure of compounds 1, 3 and 4 respectively at a concentration of 200.0 µg/ml, (Figure 4.5). IC₅₀ values for compounds 1, 3 and 4 were found to be 4.79 ± 2.54, 6.27 ± 4.75 and 5.86 ± 4.28 µg/ml respectively (Table 4.1) In literature according to Parimaladevi *et al.* (2004) betulin isolated from *Cleome viscosa* (25, 50 mg/kg) exhibited

significant hypoglycaemic activity in both normal and streptozotocin induced diabetic rats compared with that of the standard drug glibenclamide (10 mg/kg). The results obtained by Rahman *et al.* (2008) indicated that betulin obtained from the methanolic extract of the seeds of *Cichorium intybus* was inactive in inhibiting alpha-glucosidase.

Mbaze *et al.* (2007) and Rahman *et al.* (2008) found that lupeol isolated from *Fagara tessmannii* and *C. intybus* respectively did not inhibit alpha-glucosidase.

According to literature epicatechins displays antidiabetic activities and is one of the most active antioxidant constituents (Berregi *et al.*, 2003). Cho *et al.* (2006) found that catechins enhanced the expression and secretion of adiponectin, an adipocyte-specific secretory hormone that can increase insulin sensitivity and promote adipocyte differentiation. They also found that catechin treatment increased insulin-dependent glucose uptake in differentiated adipocytes and augmented the expression of adipogenic marker genes. In search of the molecular mechanism responsible for the inducible effect of (-)catechin on adiponectin expression they found that catechin suppressed the expression of Kruppel-like factor 7 protein. This protein inhibited the expression of adiponectin and other adipogenesis related genes that play an important role in the pathogenesis of type 2 diabetes. Zaid *et al.* (2002) found that treatment with epicatechin (1mM) resulted in a significant increase in the activity of erythrocyte Ca⁺⁺-ATPase in both normal and type 2 diabetic patients. According to Jalil *et al.* (2009) the intake for 4 weeks of a cocoa extract supplemented with polyphenols (2.17 mg epicatechin, 1.52 mg catechin, 0.25 mg dimer and 0.13 mg trimer g-1 cocoa extract) and methylxanthines (3.55 mg caffeine and 2.22 mg theobromine g-1 cocoa extract) significantly (P, 0.05) reduced the plasma total cholesterol, triglycerides and low-density lipoprotein cholesterol of obese-diabetic rats compared to non-supplemented animals. A study done by Kobayashi *et al.* (2000) using a rat everted sac showed that tea polyphenols consisting mainly of catechins, epicatechin gallate, epigallocatechin and epigallocatechin gallate inhibited sodium-dependent glucose transporters. This indicated that tea polyphenols interacts with sodium-dependent glucose transporters as antagonist-like molecules, possibly playing a role in controlling dietary glucose uptake in the intestinal tract.

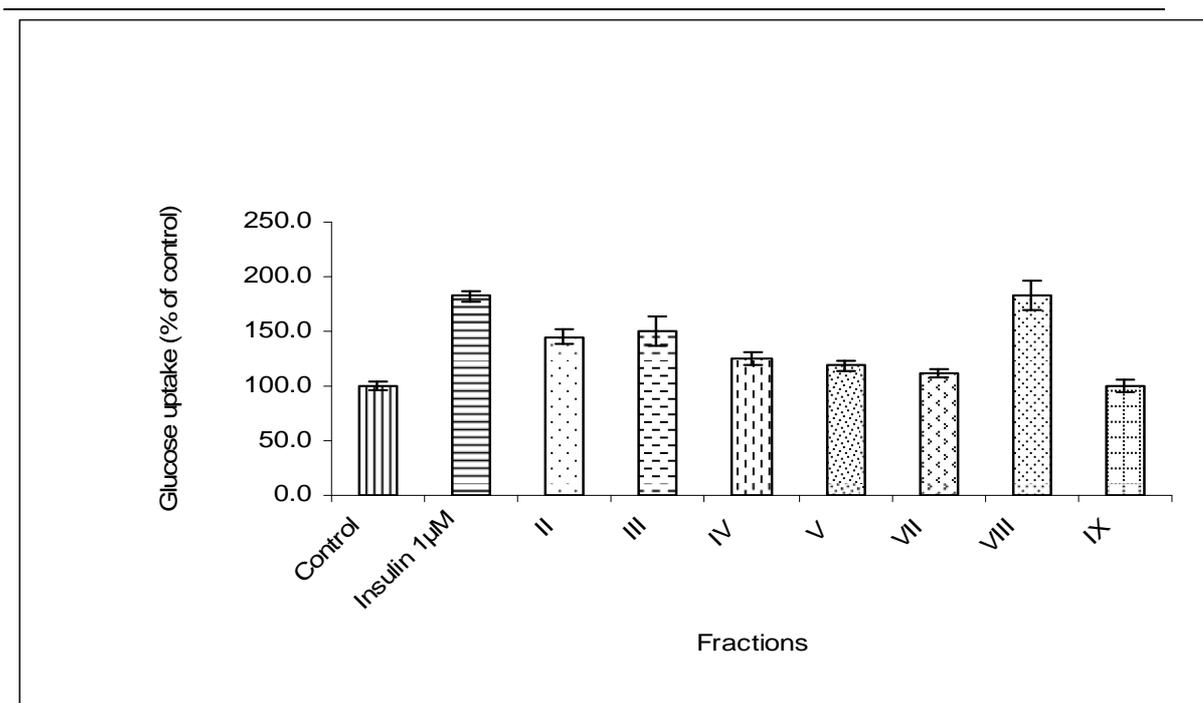


Figure 4.3. Glucose uptake (as % of control \pm standard error of mean N= 8) of the different fractions of the crude plant extract of *E. undulata* (12.5 µg/ml) tested for their hypoglycaemic activity in C2C12 myocytes

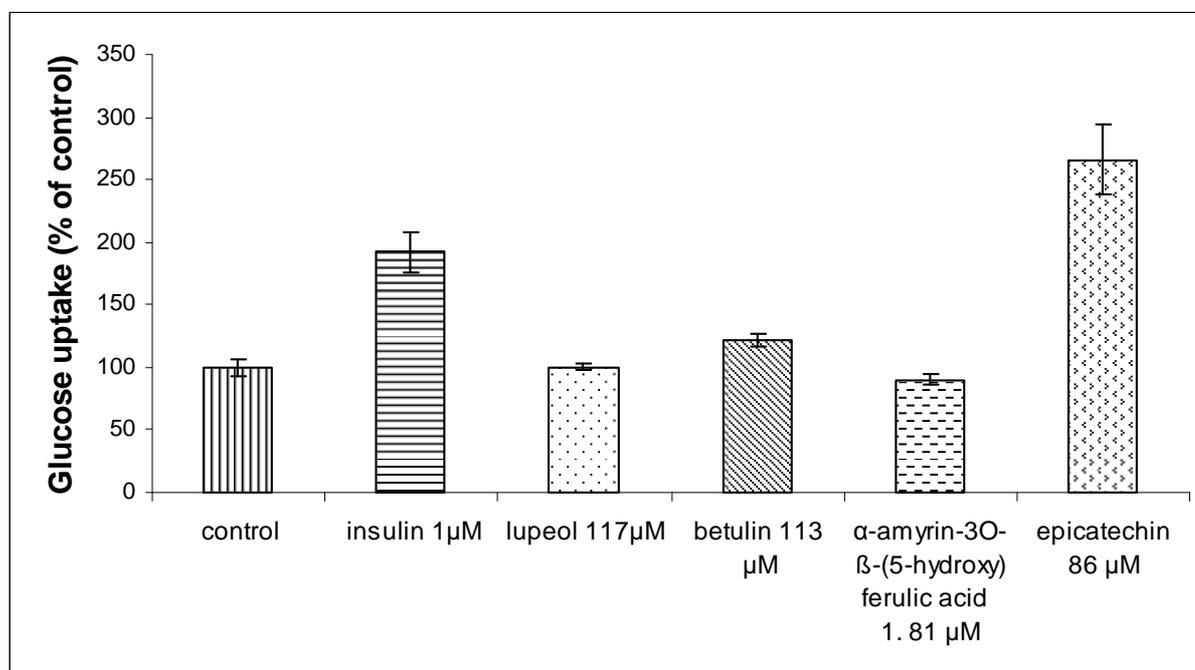


Figure 4.4. Glucose uptake (as % of control \pm standard error of mean N=8) of the compounds isolated from *E. undulata* tested for their hypoglycaemic activity using C2C12 myocytes.

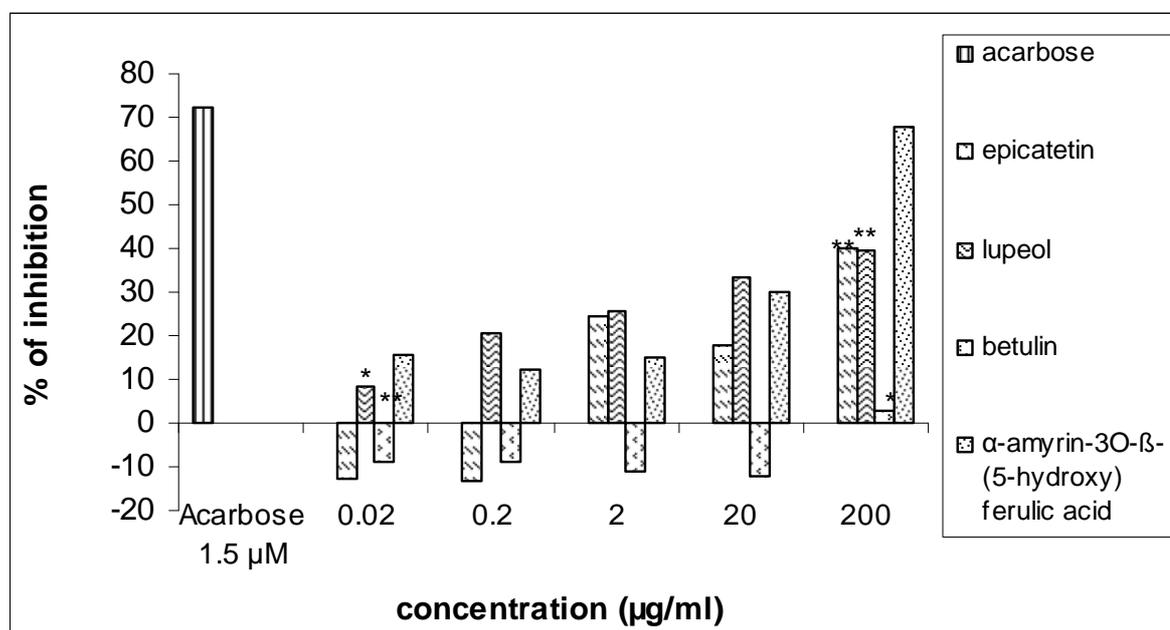


Figure 4.5. Inhibition of alpha-glucosidase by the different compounds isolated from the acetone extract of *E. undulata* (Statistic analysis were only done for the highest and lowest concentrations; * $p < 0.05$; ** $p < 0.005$; compared to control using student's two-tailed t-test)

Table 4.1 Inhibitory affect (IC_{50}) of the four compounds and positive control acarbose for alpha-glucosidase.

Compound	IC_{50} ($\mu\text{g/ml}$) alpha-glucosidase	μM
Acarbose	4.75 ± 3.18	7.35
À-amyrin-3O-β-(5-hydroxy) ferulic acid (1)	4.79 ± 2.54	7.76
Epicatechin (4)	5.86 ± 4.28	20.18
Lupeol (3)	6.27 ± 4.75	14.69
Betulin (2)	32.04 ± 2.79	72.37

4.6 Phytochemical examination

The phytochemical examination coupled with bioassay-guided fractionation of the acetone extract resulted in the isolation of a new α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**), the triterpenes: betulin (**2**) and lupeol (**3**), and the anthocyanin epicatechin (**4**) (Figures 4.1 and 4.2). Hydrolysis. A 5 mg portion of the compounds 1- 4 were added to 5ml of aqueous KOH and left under nitrogen overnight at room temperature. The reaction mixture was neutralized with 10% HCl. Compounds 1 - 4 were extracted with CHCl₃, then purified by silica gel column chromatography using 30% EtOAc in hexane.

4.6.1 Chemical constituents from the root bark of *E. undulata*

4.6.1.1 α -Amyrin-3O- β -(5-hydroxy) ferulic acid (1)

Compound (1), was purified with a Sephadex column eluted with dichloromethane and methanol, 99:1. HRESIMS showed a molecular peak at 619.47 m/z ($M^+ + H$) corresponding to the molecular formula C₄₀H₅₈O₅ (Figure 4.10). The IR spectra (Figure 4.7) revealed the presence of bands at 3362, 2945, 2870, 2359, 2341, 1700, 1643 and 1606 cm⁻¹ of α , β -unsaturated carbonyl, aromatic ring and cyclic alkane. The UV spectra gave two absorption bands at λ_{max} 228, 320 nm of the conjugated aromatic system. The ¹H NMR data of 1 (Figures 4.8) showed signals for six singlet methyls (δ_H 1.06, 1.01, 0.99, 0.92, and 0.78), and two methyl doublets (0.86, d, $J=5.6$ Hz; 0.78, d, $J=5.8$ Hz), methoxyl singlet (3.86), a methine proton bearing an ester at 4.66 (dd, $J= 10.3, 5.9$ Hz), olefinic proton at 5.10 (t, $J= 1.5$ Hz), in addition to 5-hydroxy ferulic acid signals at 7.50, 6.26 (d, $J=16.1$ Hz, α , β protons), 6.80, 6.62 (d, $J= 0.5$ Hz, H-2', 6'), the aforementioned data indicated the presence of ursane type triterpene similar to α -amyrin esterified in position C-3 with 5-hydroxy ferulic acid. The ¹³C (Figure 4.9), DEPT-135 NMR and HSQC spectra of (1) (Figure 4.12) confirmed the proposed structure of α -amyrin skeleton and showed eight methyl carbon signals, nine methylene, seven methane groups and six quaternary carbons, in addition to those signals attributed to 5-hydroxyferulic acid (Table 4.2). Thus, from the above data and comparing to the literature for similar structures (Garson *et al.*, 2006;

Nakagawa *et al.*, 2004; Ohsaki *et al.*, 2004) compound **1** was confirmed to be α -amyrin derivative esterified with 5-hydroxy ferulic acid at position C-3. The position of the methoxyl group at C-3' was deduced from the different chemical shifts of the aromatic proton H-2' and H-6' and confirmed by the HMBC (Figures 4.6 and 4.11) correlations which showed, amongst other correlations, a cross peak between MeO/C-3' and H-2'/C-3', C-4'.

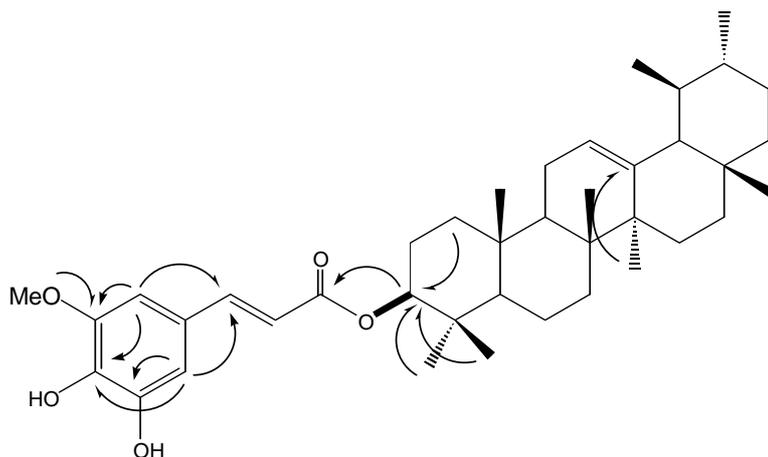


Figure 4.6 HMBC correlations of α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**).

The relative configurations of C-3 could not be determined from the recorded NOESY spectra of **1**; however, hydrolysis of **1** gave α -amyrin with a 3 β -OH configuration, which was identified on the basis of comparison of spectral data reported in literature.

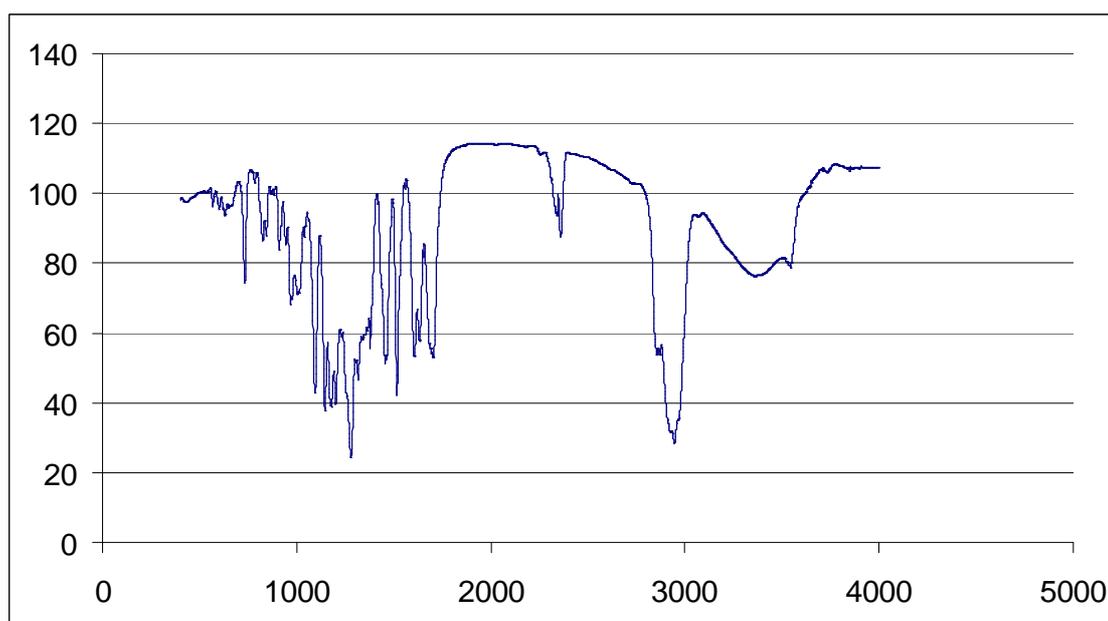


Figure 4.7 IR spectra of α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**).

Table 4.2. NMR data of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1),

No.	C	H, J Hz	No.	C	H J Hz
1	38.37	1.13 m, 1.67 m	21	31.22	1.31 m, 1.39 m
2	23.68	1.6 m	22	41.51	1.29 m, 1.46 m
3	81.04	4.66, dd, $J= 10.3, 5.9$	23	28.1	-----
4	37.9	-----	24	16.84	0.92 s
5	55.27	0.87 m	25	15.71	0.99 s
6	18.23	1.41 m, 1.52 m	26	16.89	1.01 s
7	32.84	1.33 m, 1.55 m	27	23.22	1.06 s
8	40.01	-----	28	28.71	0.78 s
9	47.52	1.55 m	29	17.48	0.78 d, $J= 5.8$
10	36.83	-----	30	20.92	0.86 d, $J= 5.6$
11	23.34	1.91 m	1'	126.6	-----
12	124.3	5.10, t, $J= 1.5$	2'	109.3	6.82 d, $J=1.5$
13	139.59	-----	3'	147.1	-----
14	42.05	-----	4'	134.8	-----
15	26.58	0.96 m, 1.83 m	5'	144.0	-----
16	28.10	0.87 m, 1.83 m	6'	103.1	6.65 d, $J=1.5$
17	33.71	-----	α	144.6	7.50 d, $J=15.9$
18	59.03	1.31 m	β	116.67	6.25 d, $J=15.9$
19	39.62	0.93 m	C=O	167.23	-----
20	39.78	1.31 m	OMe	56.20	3.86 s

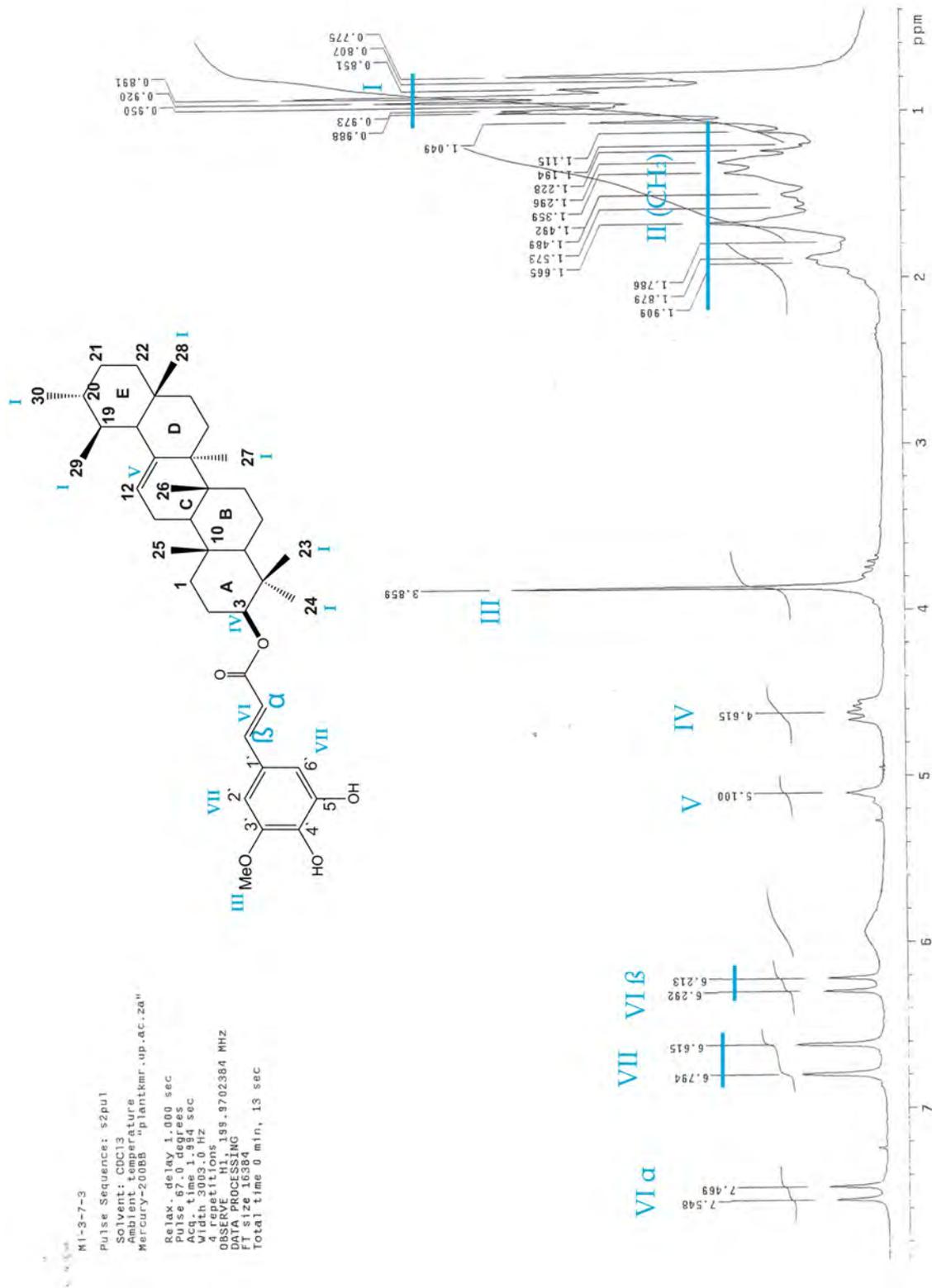


Figure 4.8 The ^1H NMR spectrum of α -amyrin-3-O- β -(5-hydroxy) ferulic acid (1)

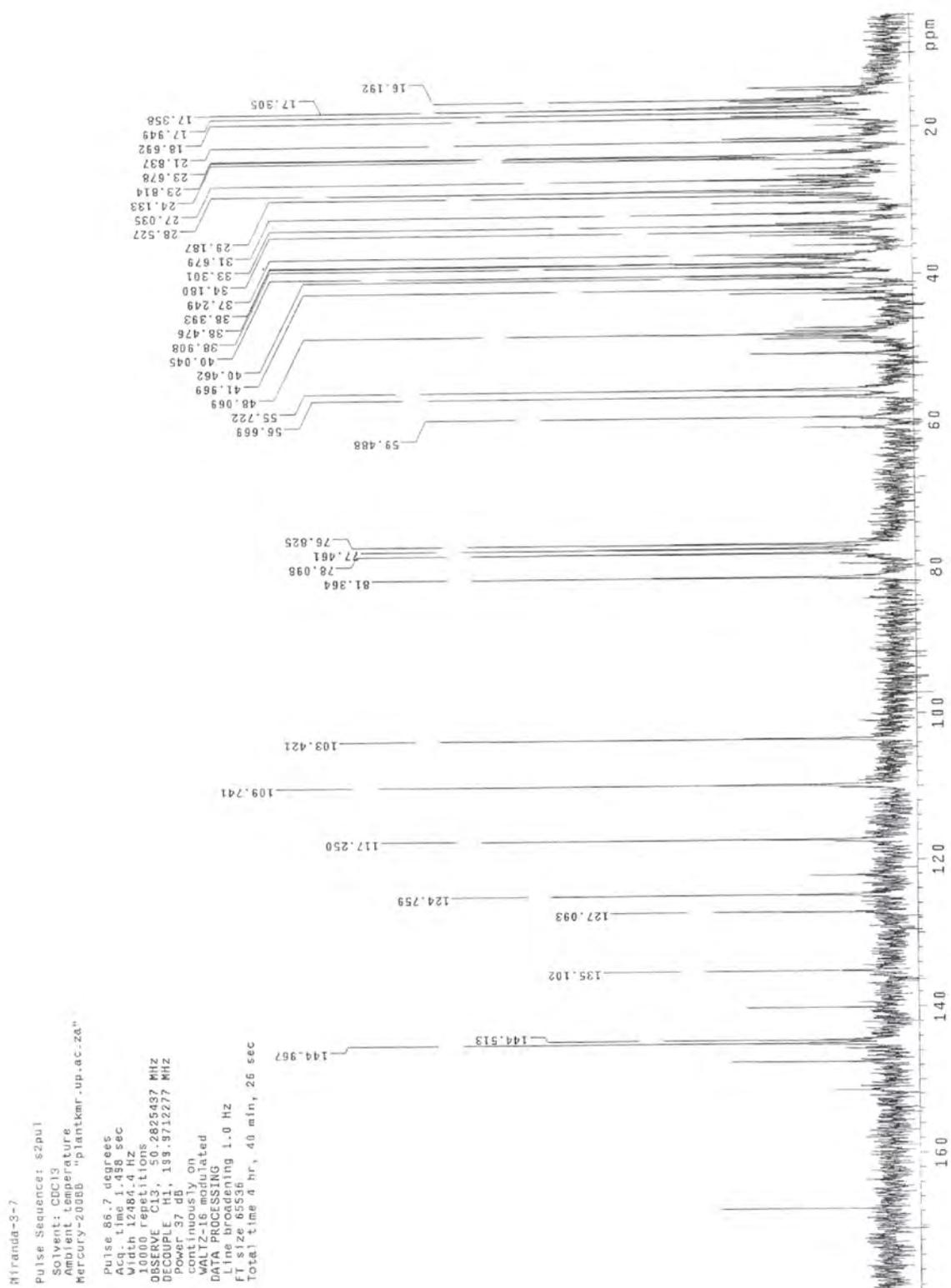


Figure 4.9 The ^{13}C NMR spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)

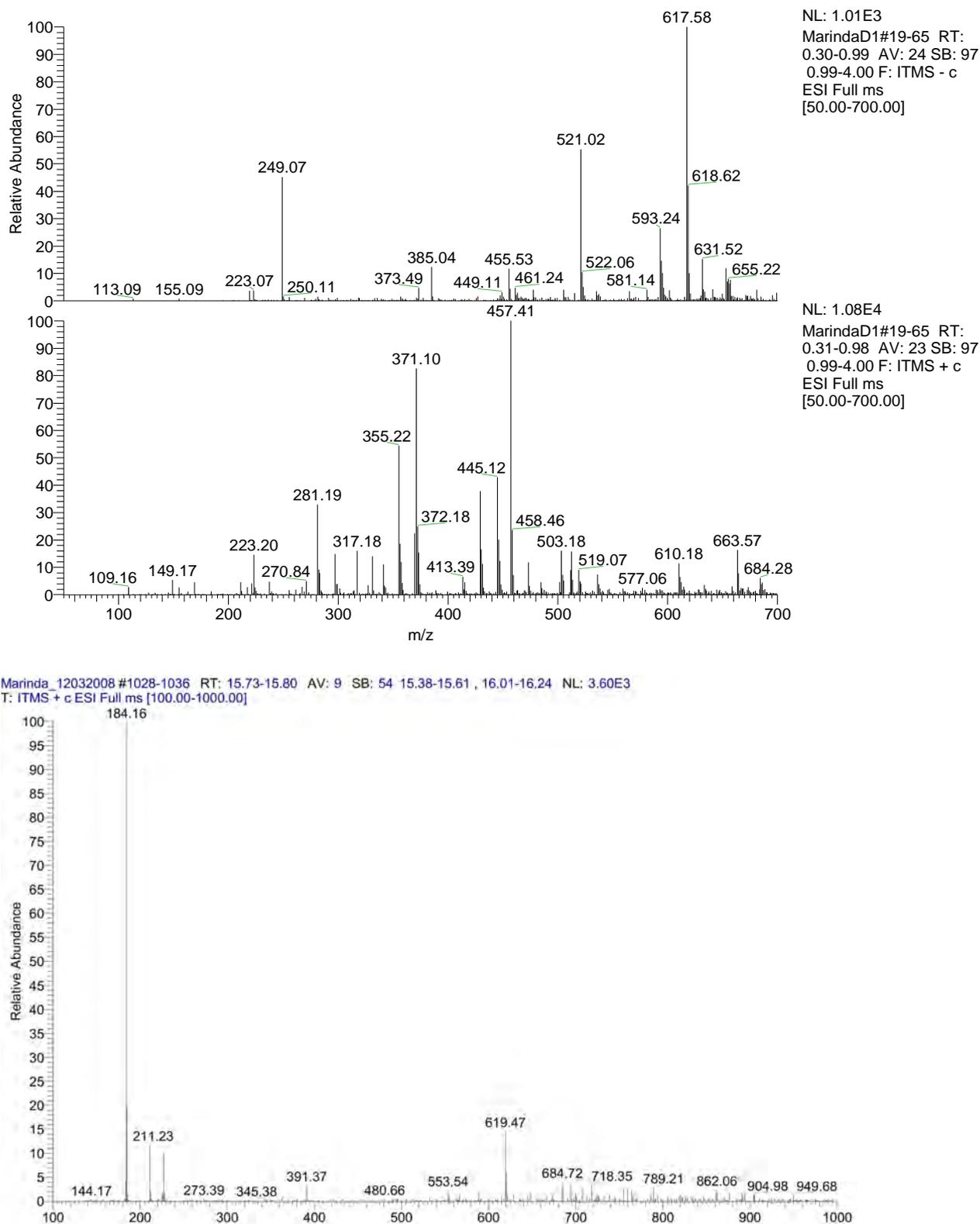


Figure 4.10. - + ESI spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)

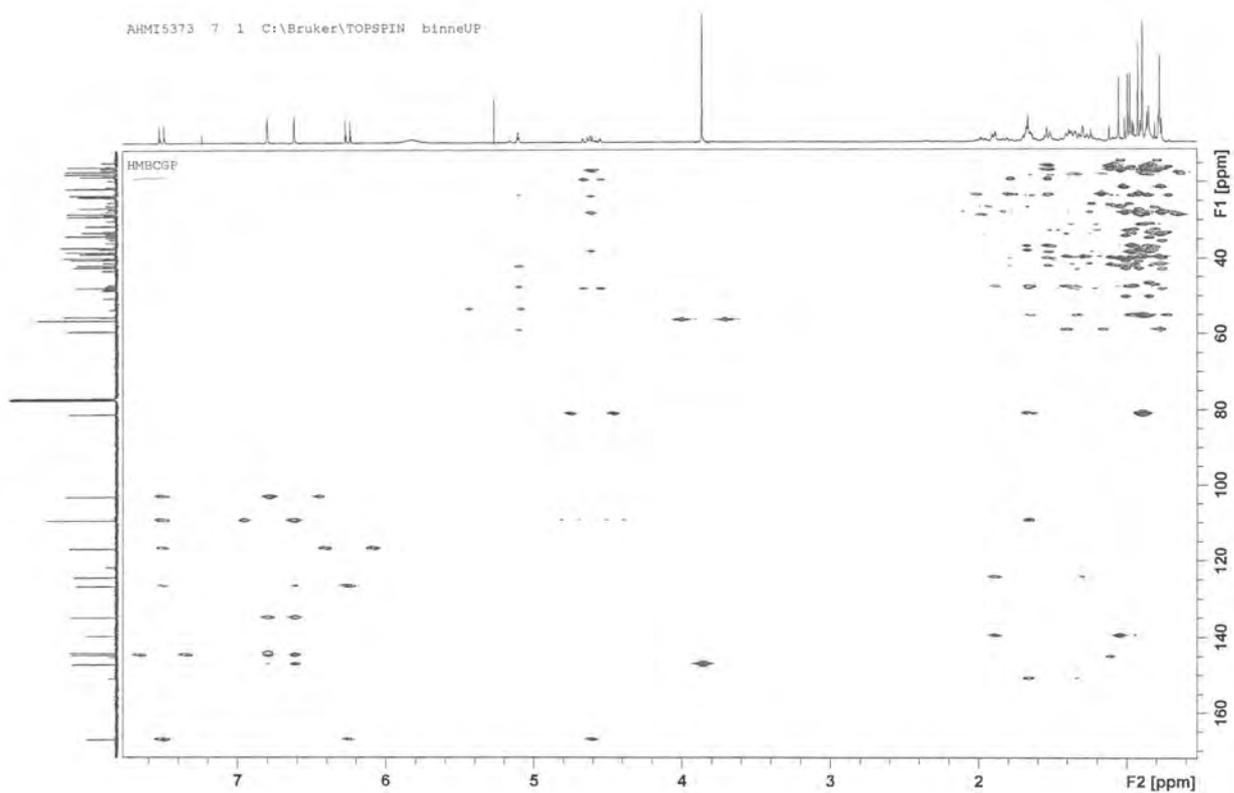


Figure 4.11. The HMBC spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)

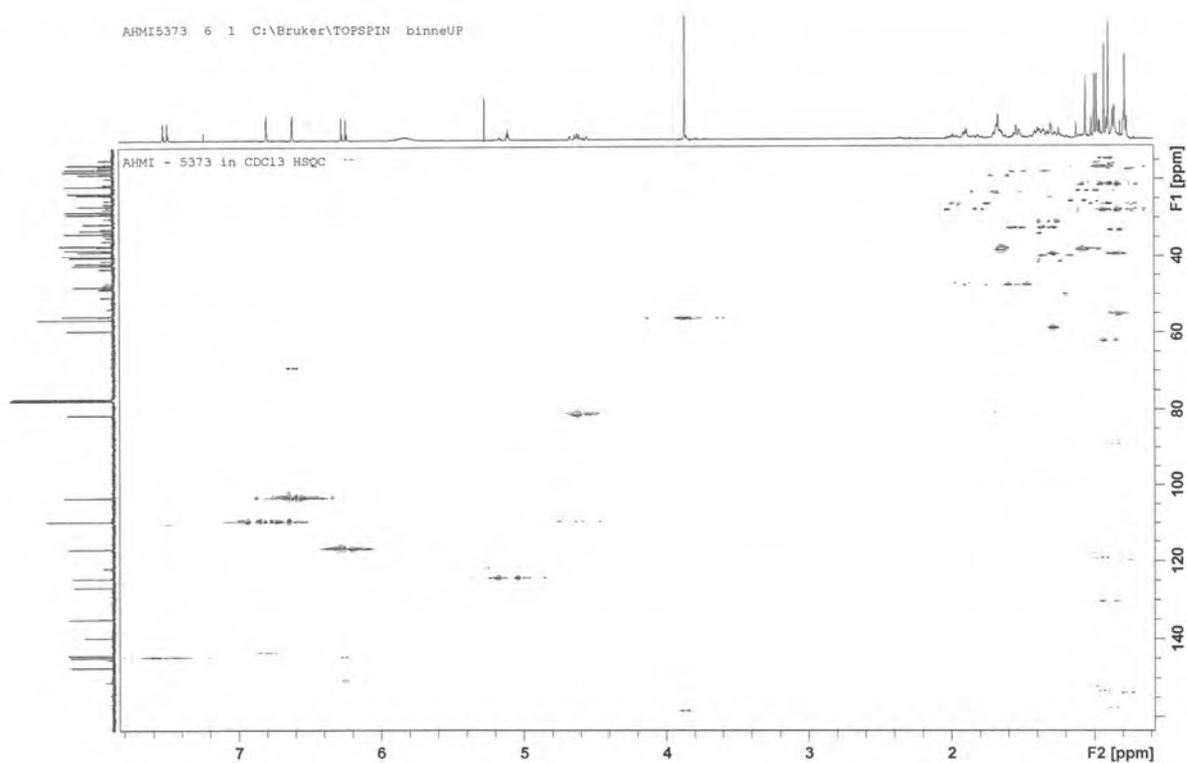


Figure 4.12. The HSQC spectrum of α -amyrin-3O- β -(5-hydroxy) ferulic acid (1)

4.6.1.2 *Betulin* (**2**)

Fraction 3 yielded a white powdery substance. The ^1H NMR data of **2** exhibited signals characteristic of the skeleton lupene triterpene, (Figure 4.13). Five signals of three proton singlets at δ 0.75, 0.81, 0.92, 0.96, 1.01 and 1.67 ppm were obtained as well as one proton signal at δ 2.4, ddd [H-19], one proton at δ 3.2, dd [H-3 α] and two proton signals at δ 4.57, 4.66 ppm. The disappearance of one methyl group from the lupeol skeleton and appearance of two protons signal at 3.32, 3.77 ppm (d, each 1H, J=10.8 Hz) indicated the oxidation of this methyl group which is characteristic of the triterpene betulin which was isolated from the Ebenaceae family before by Mallavadhani *et al.* (1998) and Weigenand *et al.* (2004). Betulin was isolated from various plant species including *Cleome viscosas* (Parimaladevi *et al.*, 2004) and from the root bark of *Euclea natalensis* (Weigenand *et al.*, 2004), but this is the first report of the isolation of betulin from *E. undulata*.

4.6.1.3 *Lupeol* (**3**)

Compound **3** was eluted from fraction 2 and identified as lupeol based on the ^1H NMR data (Figure 4.14).

The ^1H -NMR data exhibited seven signals of three protons at δ 0.75, 0.78, 0.82, 0.94, 0.96, 1.02 and 1.67 ppm. The proton signal at 2.39 ppm, ddd, was assigned to H-19, the signal at δ 3.2 ppm, dd, to 3 α -H, and two proton signals at δ 4.55 and 4.68 ppm of H-29A, 29B. One shows long range coupling with one methyl at δ 1.67. The last two protons are assigned to isopropenyl olefinic protons.

The ^1H -NMR data indicated that the compound isolated is the commonly found triterpene, lupeol, which had previously been isolated from *E. natalensis* by Khan & Rwekika (1992) and Weigenand *et al.* (2004). Lupeol has been isolated from various plant species including *Spirostachys africana* (Mathabe *et al.*, 2008) and from the root bark of *E. natalensis* (Weigenand *et al.*, 2004), but this is the first report of the isolation of lupeol from *E. undulata*.

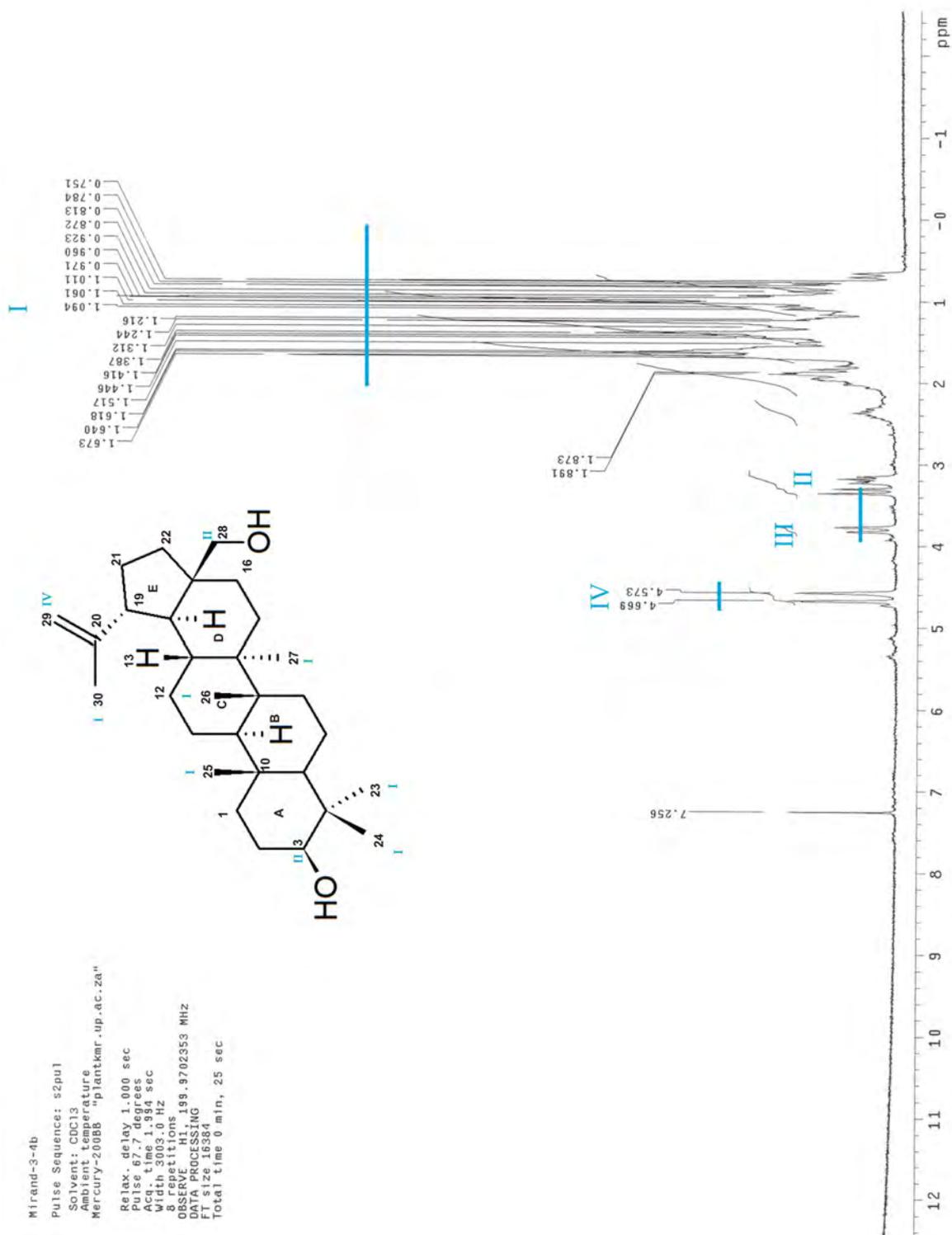


Figure 4.13. ¹H NMR spectrum of betulin (2)

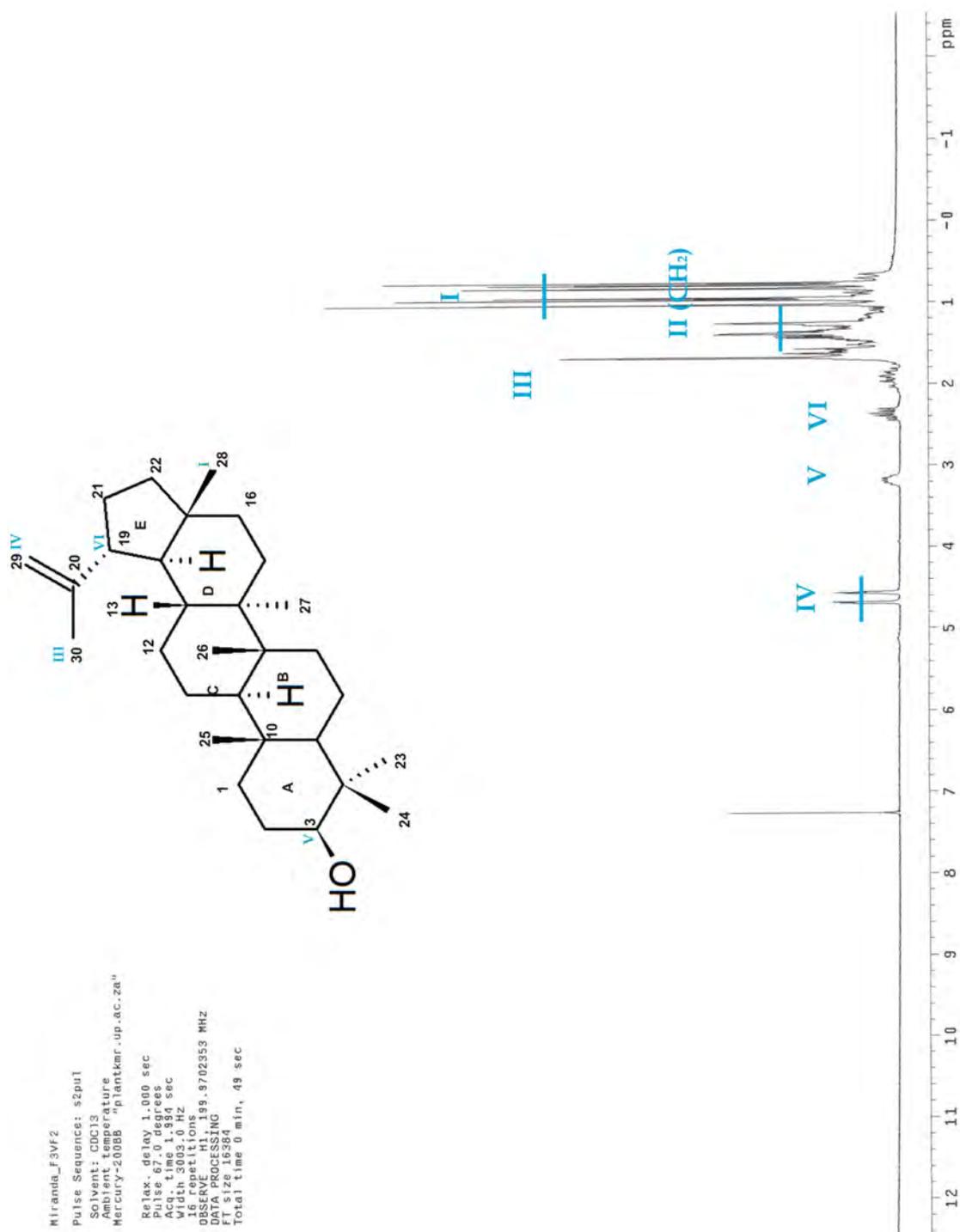


Figure 4.14. ¹H NMR spectrum of lupeol (3)

4.6.1.4. Epicatechin (**4**)

A reddish brown powdery substance was eluted from fraction 8 and identified on the basis of $^1\text{H-NMR}$ data (Figure 4.15). The $^1\text{H-NMR}$ data of **4** exhibited signals identical with that of epicatechin. The $^1\text{H NMR}$ data exhibited signals of 6 protons, at δ 4.85 assigned to H-2, a proton signal at δ 4.18 ppm, to H-3, and two proton signals at δ 2.74 and 2.80 ppm were assigned to protons 4 α and 4 β respectively, signal at δ 6.01 was assigned to H-6 and the signal at 5.90 to H-8 (Okushio *et al.* 1998). Aromatic signals at δ H 7.02, 6.75 and δ H 6.80 corresponds with that of a B-ring. The basic structure was derived as a 3,3',4',5,7-pentahydroxyflavan and the broad proton singlet at δ H 4.82 suggested a epicatechin (Figure 4.15) (Sun *et al.* 2006).

In vitro assays on C2C12 myocytes were done on the four compounds isolated from the root bark of *E. undulata* to determine their hypoglycaemic activity as well as their alpha-glucosidase inhibition potential.

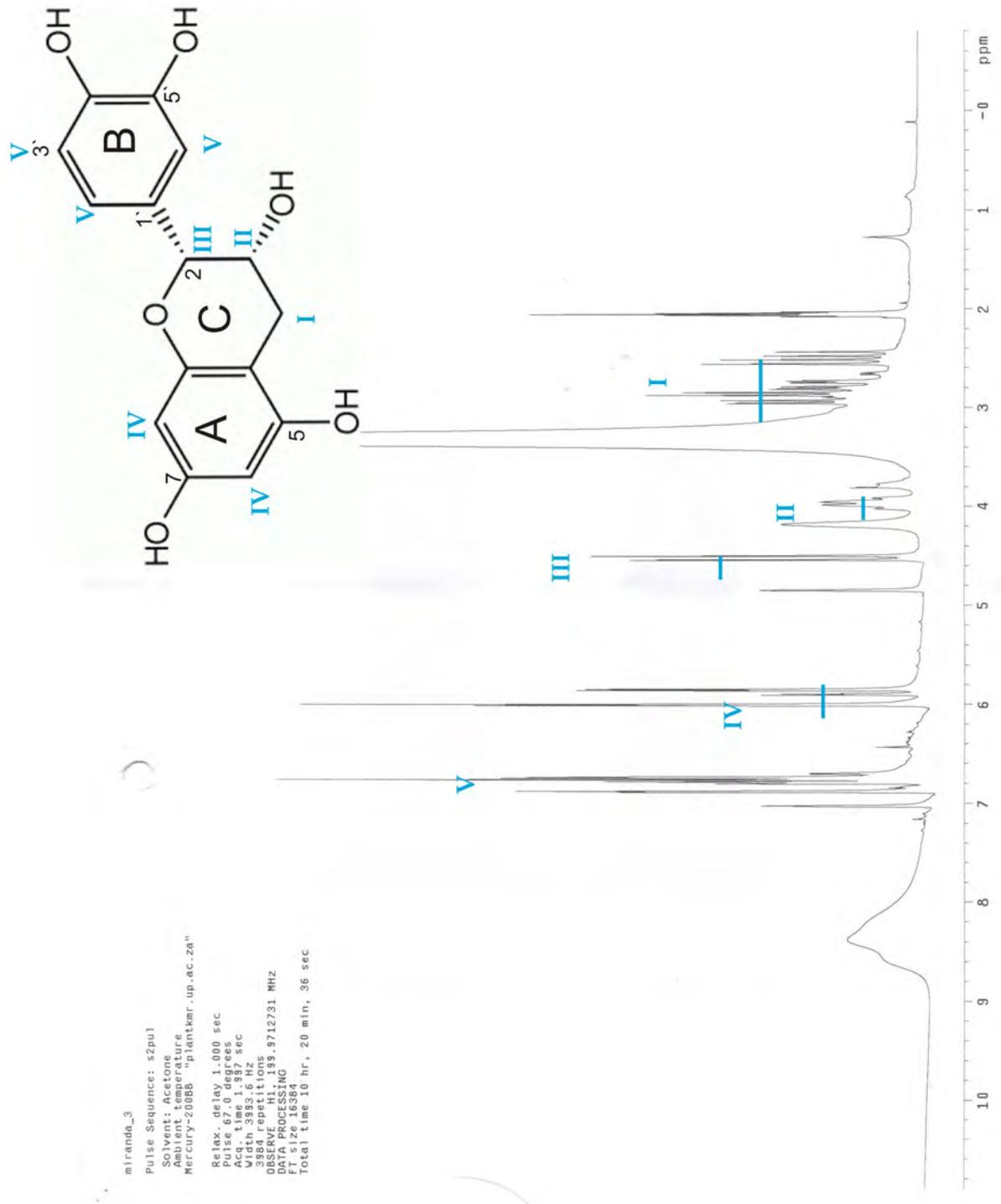


Figure 4.15. ¹H-NMR spectrum of epicatechin (4)

4.7. Conclusions

Phytochemical studies conducted on *Euclea* species by Orzalesi *et al.*, (1970-71) and Costa *et al.* (1978) demonstrated the presence of triterpenoids in the stems and leaves. Two naphthoquinones, diospyrin and 7-methyl-juglone, were isolated from the root, stem and fruit of *E. undulata* var. *myrtina* by Van der Vyver *et al.* (1973; 1974). Chemical analysis indicated the presence of 3.26 % tannins in bark, saponins and reducing sugars in leaves and stems, but no alkaloids, naphthoquinones or cardiac glycosides (South African National Biodiversity Institute, 2005). In this study it seemed as if the root bark of *E. undulata* var. *myrtina* was devoid of naphthoquinones. These contradicting findings may be attributed to the extraction procedures and different environmental factors such as geographical and seasonal variation. Unfortunately the localities and time of collection by Van der Vyver and Gerritsma, (1973; 1974) could not be established. Khan (1985) reported that the relative amounts of 7-methyljuglone and lupeol in *E. natalensis* are season dependent and interrelated and could indicate some biogenetic relationship between the two natural products.

It was reported in literature that aqueous leaf extract of *E. undulata* demonstrated antimicrobial activity *in vitro*, at a concentration of 40 mg/ml, against *Staphylococcus aureus*. This result, together with the presence of tannins in the leaves, supports its use as anti-diarrhoeal and for the relief of tonsillitis. No activity against *Pseudomonas aeruginosa*, *Candida albicans* or *Mycobacterium smegmatis* was shown in the preliminary tests (South African National Biodiversity Institute, 2005).

The phytochemical examination coupled with bioassay-guided fractionation of the crude acetone extract of the root bark of *E. undulata* var. *myrtina* afforded a new triterpene and three other known compounds **1-4**. The results obtained from the *in vitro* assays on the main fractions with C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells indicated that three of the main fractions showed anti-diabetic activity. The identified main fractions were sub-sequently subfractionated and four compounds isolated; α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**), betulin (**2**), lupeol (**3**) and epicatechin (**4**). These compounds,

isolated for the first time from *E. undulata* var *myrtina*, were evaluated for, their hypoglycaemic activities by executing *in vitro* assays on C2C12 myocytes, as well as their ability to inhibit the carbohydrate-hydrolysing enzyme alpha-glucosidase. The present study reports for the first time the alpha-glucosidase inhibitory activity and glucose utilization by C2C12 myocytes of an acetone extract of *E. undulata* and its purified compounds.

The results indicated that epicatechin has the ability to lower blood glucose levels, whereas α -myrin-3O- β -(5-hydroxy) ferulic acid has the ability to inhibit alpha-glucosidase. These findings corroborate the ethnomedicinal use of *E. undulata* by traditional healers for the treatment of diabetes.

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GENERAL DISCUSSION AND CONCLUSIONS

Diabetes mellitus comprises a collection of heterogeneous diseases that differ in their etiological, clinical, and epidemiological characteristics, but have hyperglycaemia and glucose intolerance in concurrence, which are either due to insulin deficiency or to the impaired effectiveness of insulin's action or a combination of both (Roussel, 1998). Five major categories of diabetes mellitus are identified, namely; insulin dependent diabetes mellitus (IDDM, type 1 diabetes), non-insulin dependant diabetes (NIDDM, type II diabetes), impaired glucose tolerance, gestational diabetes mellitus and undiagnosed diabetes mellitus (Szava-Kovats and Johnson, 1997). The two dominant types are insulin dependent diabetes mellitus and non-insulin dependant diabetes mellitus. It is a chronic disease with major long-term implications, not only for the health and well-being of affected individuals, but also for costs to the society as a whole (Szava-Kovats and Johnson, 1997). As a chronic metabolic disorder, diabetes mellitus can affect all the body's major organ systems leading to complications that are a source of significant morbidity and premature mortality, making it a costly disease (Szava-Kovats and Johnson, 1997). According to the World Health Organization it will affect an estimated 366 million people in 2030 (Motala *et al.*, 2008).

Until the 1980s, the few reported studies on diabetes in Africa indicated a low prevalence of diabetes that is between 0 and 1.0 % in sub-Saharan Africa. However, over the past few decades, type 2 diabetes has emerged as an important medical problem in this region (Motala *et al.*, 2008). Recent estimates by the International Diabetes Federation indicated that the largest increase in the prevalence of diabetes is expected to occur in developing regions of the world, including Africa (Motala *et al.*, 2008). The projected increase in diabetes for Africa is from 3.1% in 2007 to 3.5% in 2025 with the corresponding increase in numbers from 10.4 to 18.7 million (International Diabetes Federation, 2006). Currently there are approximately 6.5 million diabetics in South Africa (Health 24, 2006).

Diabetes is a growing concern as African populations become Westernized, urbanized and adopt a Western diet that often leads to overweight and obesity. It was the sixth leading

natural cause of death in South Africa for the 2004 – 2005 period (South Africa Government Information, 2007).

Unfortunately, there is no cure yet for diabetes, but by controlling blood sugar levels through a healthy diet, exercise and medication, the long term complications of diabetes can be minimized. The progressive nature of the disease necessitates constant reassessment of glycaemic control in people with diabetes, and the appropriate adjustment of therapeutic regimes when glycaemic control is no longer maintained with a single agent. The addition of a second and third drug is usually more effective than switching to another single agent (Gerich, 2001).

The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes and primary health care. A large portion of the population relies heavily on traditional healers and herbalist to meet primary health care needs. Although modern medicine may be available, herbal medicine has often maintained popularity for cultural and historical reasons. Four plant species, *S. pinnata*, *E. transvaalense*, *P. divaricata* and *E. undulata*, used for the treatment of diabetes by traditional healers and herbalist in South Africa, were validated for their hypoglycaemic activity and toxicity. The plant species were investigated by executing *in vitro* assays for hypoglycaemic, alpha-glucosidase and alpha-amylase, as well as, C2C12 myocyte, 3T3-L1 preadipocyte and Chang liver cell on the various plant extracts.

The *in vitro* antidiabetic screening of the four acetone and single ethanol plant extracts was carried out using a method developed by Van de Venter *et al* (2008). This method measures glucose utilization, which can be used for long-term exposure of the cells to the sample.

The alpha-glucosidase inhibiting activity of the four acetone plant extracts were tested by making use of the 96-well microplate assay developed by Collins *et al*. (1997). Alpha-glucosidase inhibitory activity was determined by measuring the release of *p*-nitrophenol from *p*-nitrophenyl- α -D-glucopyranose. The released *p*-nitrophenol yielded a yellow colour when the stopping reagent glycine was added.

. An adapted method described by Park and Johnson (1949), Bernfeld (1955) and Slaughter *et al.* (2001) was used to determine the alpha- amylase inhibiting activity of the four acetone plant extracts. The reduction of ferricyanide ions in alkaline solutions followed by the formation of Prussian blue (ferric ferrocyanide) was measured quantitatively as a basis for the estimation of glucose levels.

The alpha-glucosidase inhibitory activity for the four acetone plant extracts at five different concentrations demonstrated that the percentage of inhibition is concentration dependent. Three of the four acetone plant extracts namely *E. undulata*, *E. transvaalense* and *P. divaricata* inhibited alpha-glucosidase while *S. pinnata* showed no inhibition.

The *in vitro* assays in C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells indicated that four of the five plant extracts tested, namely *S. pinnata* (ethanol/ acetone), *E. undulata* (acetone) and *E. transvaalense* (acetone), showed positive results in increasing glucose utilization whereas *P. divaricata* showed no ability. The cytotoxicity tests, however, revealed that the *S. pinnata* extracts (ethanol/acetone) were toxic to 3T3-L1 preadipocytes and that the *E. transvaalense* extract was toxic to Chang liver cells. These results were interpreted by making use of the scoring system developed by Van de Venter *et al.* (2008). According to this system *E. undulata* scored a +3 out of a maximum activity score of +6 and was therefore chosen for further analysis.

The crude acetone extract (35 g) of *E. undulata* was subjected to silica-gel column chromatography for the isolation of bioactive principals. Fractions containing the same compounds as determined by thin layer chromatography (TLC) were combined. Nine main fractions were obtained. Fraction II was chromatographed over a silica column and yielded (2500 mg; 71.4% yield) lupeol (**3**). Fractions III and IV were combined and chromatographed over a sephadex column using ethanol and yielded a new α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**) (14.28 mg; 0.40% yield) and betulin (**2**) (20.01 mg; 0.57% yield). Fraction VIII was chromatographed over a sephadex column and yielded (12.02 mg; 0.34% yield) epicatechin (**4**).

An *in vitro* assay was executed on C2C12 myocytes on the four isolated compounds and revealed that lupeol (**3**) and α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**) were inactive in lowering blood glucose levels, whereas betulin (**2**) was slightly active, and epicatechin (**4**) was active in lowering blood glucose levels. The alpha-glucosidase assay on α -amyrin-3O- β -(5-hydroxy) ferulic acid (**1**), betulin (**2**), lupeol (**3**), and epicatechin (**4**) indicated that (**1**) inhibited alpha-glucosidase.

In conclusion, the results obtained from the various assays executed on the four plant extracts screened validated the use of all four plant extracts for the treatment of diabetes by traditional healers and herbalists. This conclusion was drawn as all the different plant extracts had the potential to lower blood glucose levels or to inhibit alpha-glucosidase. The assays, however, also revealed the cytotoxicity of the *S. pinnata* extracts to 3T3-L1 preadipocytes and of *E. transvaalense* to Chang liver cells, indicating that these plants should be used with caution in the treatment of diabetes. The application of different assays revealed that different plant extracts may function in different ways to lower blood glucose levels as indicated by the *P. divaricata* extract. No activity was detected in the C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cell assays, but it inhibited alpha-glucosidase and alpha-amylase to some extent.

The fractionation of the crude acetone plant extract of *E. undulata* led to the isolation, for the first time, of four compounds from *E. undulata*, namely; a new α -amyrin-3O- β -(5-hydroxy) ferulic acid, betulin, lupeol and epicatechin. The consequent hypoglaecamic assays disclosed that the isolated epicatechin has the potential to lower blood glucose levels and the newly isolated α -amyrin-3O- β -(5-hydroxy) ferulic acid has the potential to inhibit alpha-glucosidase. The present study is, according to our knowledge, the first report on the alpha-glucosidase inhibitory activity and glucose utilization in C2C12 myocytes of the crude acetone extract of *E. undulata* and its purified compounds.

Positive results were obtained from this study. It is however, recommended that further assays be performed on the four plant species evaluated for their hypoglycaemic activity and toxicity. This should be done by using an aqueous extracts as used by traditional healers and herbalists. It is also of great concern that in this study none of the

naphthoquinones, nor diospyrin or 7-methyl-juglone were isolated from the root bark of *E. undulata*. It is possible that the seasonally dependent interrelationship between the amount of 7-methyljuglone and lupeol reported by Khan (1985) in respect of *E. natalensis* may also be true for *E. undulata*. It is therefore necessary that this interrelationship be investigated as juglone is toxic (Thiboldeau *et al.* 1994; Ganapaty *et al.* 2004 and Kong *et al.* 2008)

It is also recommended that the extract of *E. undulata* should be tested *in vivo* in a rat or mouse model for its hypoglycaemic activity as the activity of some of the plant extracts may differ *in vitro* and *in vivo*. The purified compounds should be tested for their inhibition of the enzyme alpha-amylase and not just for alpha-glucosidase. Due to time constraints and insufficient purified compounds the above mentioned test could not be performed.

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My Creator for giving me this opportunity and strength.

6.1 Congress attended

Deutschländer, M.S., Lall, N., Van de Venter, and S. Roux. 2006. Detection of anti-diabetic activity in South African plant extracts. IPUF. Botswana. July 2006.

Deutschlander, M.S., Lall, N. and Van de Venter. 2010. Isolation and identification of a novel anti-diabetic compound from *Euclea undulata* Thunb. SAAB. University of the North West, North West Province, South Africa. January 2010.

6.2 Publications from this thesis

Deuschländer, M.S., Lall, N. and Van de Venter, M. 2009. Plant species used in the treatment of diabetes by South African traditional healers: an inventory. *Pharmaceutical Biology*. 47 (4): 348 - 365

Deuschländer, M.S., Van de Venter, M. Roux, S, Louw, J. and Lall, N. 2009. Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology*. 124 (3): 619-624

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Appendix 1: Colour photographs of the plant species described in Chapter 2



Figure 1. *Elaeodendron transvaalense* (Van Wyk et al., 2000.)



Figure 2. *Euclea undulata* (Lizande Kellerman)



Figure 3. *Euclea natalensis* (Namrita Lall)



Figure 4. *Lannea edulis* (Van Wyk et al., 2005)

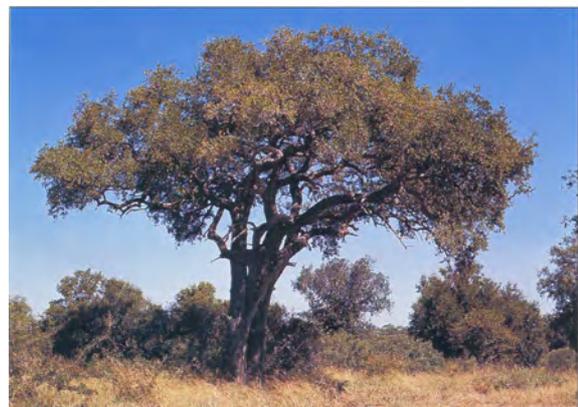


Figure 5. *Spirostachys africana* (Van Wyk et al., 2000)



Schkuhria pinnata

Figure 6. *Schkuhria pinnata* (Bromilow, 1995).



Figure 7. *Pteronia divaricata*
(De Villiers, 1999)



Figure 8. *Ziziphus mucronata* (Van Wyk *et al.*, 2000)



Figure 9. *Aloe ferox*
(Van Wyk & Smith, 1996)



Figure 10. *Warburgia salutaris* (Van Wyk *et al.*, 2000)

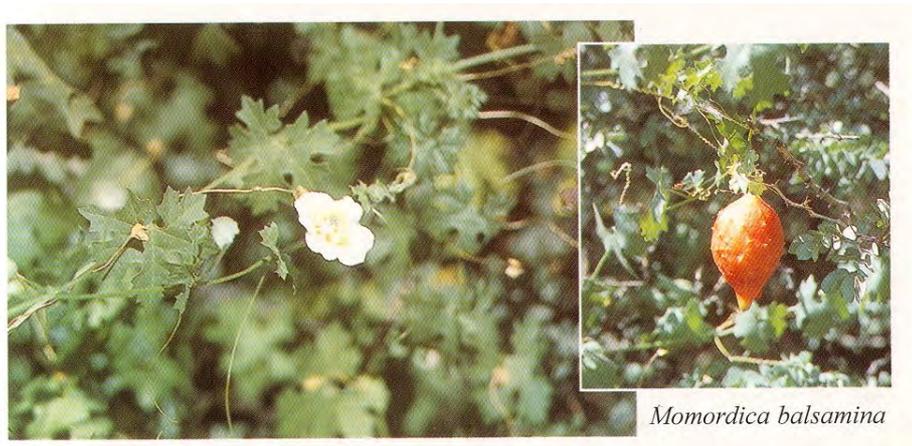


Figure 11. *Momordica balsamina* (Van Rooyen, 2001)



Figure 12. *Kedrostis nana* (Manning, 2000: Van Wyk & Gericke, 2000)

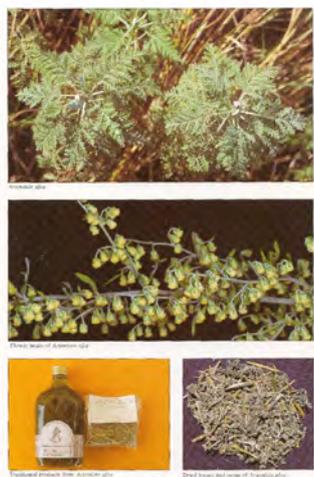


Figure 13. *Artemisia afra* (Van Wyk *et al*,2005).



Figure 14. *Catharanthus roseus*



Figure 15. *Cnicus benedictus* (Van Wyk *et al.*, 2005)



Figure 16. *Psidium guajava*
(Van Wyk *et al.*, 2005)



Figure 17. *Terminalia sericea* (Van Wyk *et al.*, 2000)



Figure 18. *Sutherlandia frutescens*
(Van Wyk *et al.*, 2005).

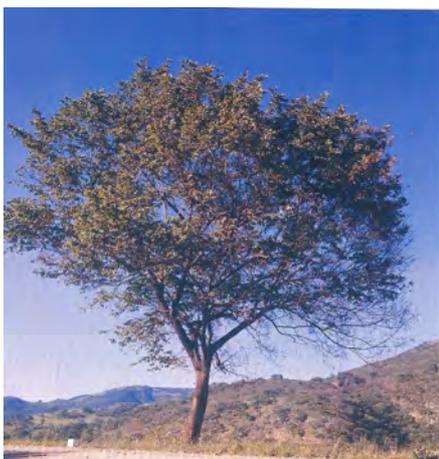


Figure 19. *Bridelia micrantha* (Van Wyk *et al.*, 1997).



Figure 20. *Sclerocarya birrea* (Van Wyk *et al.*,
2000).



Figure 21. *Brachylaena discolor* (Schmidt *et al.* 2002).



Figure 22. *Brachylaena elliptica* (Pooley, 1997).



Figure 23. *Brachylaena ilicifolia* (Schmidt *et al.*, 2002) (Pooley, 1997)



Figure 24. *Bulbine natalensis* (Van Wyk *et al.* 2005)



Figure 25. *Carpobrotus edulis* (Smith., et al., 1998).



Figure 26. *Chironia braccifera*
(Van Wyk et al., 2005).



Figure 27. *Cissampelos capensis* (Van Wyk et al., 2005)



Figure 28. *Harpagophytum procumbens* (M. Ströbach)



Figure 29. *Hoodia currorii* (Van Wyk & Gericke, 2000).

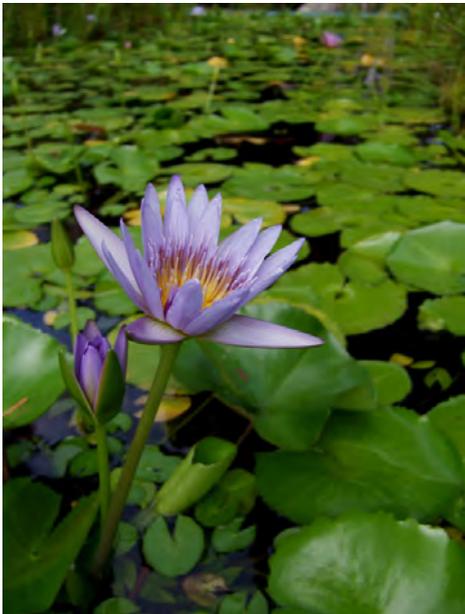


Figure 30. *Nymphaea caerulea* var. *caerulea*
[http://www.ubcbotanicalgarden.org/potd/2007/06/nymphaea_nouchali_var_caerulea.p] (2008/12/08).



Figure 31. *Trigonella foenumgraecum*
(HerbCD PhytoPharm, 1996. Berlin, Germany)

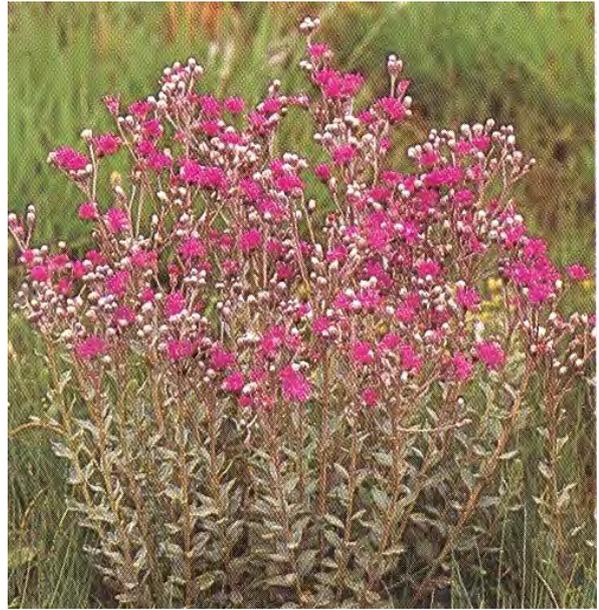


Figure 32. *Vernonia oligocephala*
(Van Wyk & Malan, 1988).