ANTIDIABETIC COMPOUNDS FROM MEDICINAL PLANTS USED IN THE INDIGENOUS SYSTEM OF MEDICINE ('DESHIYA CHIKITSA') IN SRI LANKA

M. Malitha Aravinda Siriwardhene

Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Pharmacology in August 2014

DOI: 10.31357/fmesmph.2015.00107
We certified that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.

Dr. A. K. E. Goonetilleke

Date 15/06/2015

Dr. G. A. Sirimal Premakumara

Date 15/06/2015
DECLARATION

"The work described in this thesis was carried out by me under the supervision of Dr. A. K. E. Goonetilleke, Senior Lecturer, Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda and Dr. G. A. Sirimal Premakumara, Research fellow and former Director, Industrial Technology Institute, Colombo 7 and a report on this has not been submitted in whole or in part to any university or any other institution for anther Degree/Diploma".

15/06/2015

Date

Signature
"I certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation".

Date: 15/06/2015

Dr. A. K. E. Goonetilleke
Senior Lecturer.
Dept. of Pharmacology,
Faculty of Medical Sciences,
University of Sri Jayewardenepura,
Nugegoda.
"I certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation".

Date
15/06/2015

Dr. G. A. Sirimal Premakumara
(Research Fellow)
# CONTENTS

| LIST OF TABLES | vi |
| LIST OF FIGURES | ix |
| LIST OF ABBREVIATIONS | xii |
| ACKNOWLEDGEMENTS | xiv |
| ABSTRACT | xvi |

## 1.0 INTRODUCTION

1.1 Diabetes Mellitus and blood glucose homeostasis ............ 1

1.1.1 Diabetes Mellitus ....................................... 1

1.1.2 The Classification of Diabetes Mellitus .................. 2

1.2 Prevalence of Diabetes Mellitus in Sri Lanka ................. 3

1.3 Animal models in anti-diabetic evaluation .................... 4

1.4 Oral hypoglycemic agents .................................. 8

1.5 Phytomedicines in Diabetes Mellitus ........................ 16

## 2.0 LITERATURE REVIEWS

2.1 Anti-diabetic ethno-medicine in Sri Lanka .................... 19

2.2 *Costus speciosus* Linn .................................... 20

2.3 *Passiflora foetida* Linn .................................... 24

2.4 *Ficus racemosa* Linn ..................................... 27

2.5 *Osbeckia octandra* Linn .................................... 31
3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents ................................................ 36
3.1.2 Kits ........................................................................ 37
3.1.3 Facilities .................................................................. 37
3.1.4 Animals .................................................................... 37

3.2 Methods

3.2.1 Survey on anti-diabetic medicinal plants used in Sri Lanka .... 38
3.2.2 Plant material ............................................................ 39
3.2.3 Extraction of plant material .......................................... 39
3.2.4 Bioactivity guided solvent partitioning of 80% methanol extracts ......................................................... 40
3.2.5 Preliminary phytochemical screening of 80% methanol extracts ......................................................... 43

i. Test for alkaloids .......................................................... 43
ii. Test for steroidal compounds ........................................ 44
iii. Test for phenolic compounds ......................................... 45
iv. Test for flavonoids ...................................................... 45
v. Test for saponins .......................................................... 46
vi. Test for tannins .................................................... 47
vii. Test for anthraquinones .......................................... 48

3.2.6 Quantitative determination of total phenols ....................... 49
3.2.7 Spectrophotometric determination of total alkaloids ............. 49
3.2.8 Spectrophotometric Determination of saponins .................. 50
3.2.9 Preliminary hypoglycemic activity of aqueous extracts .......... 52
3.2.10 Evaluation of anti-hyperglycemic activity in glucose loaded
        Normal Wistar rats ..................................................... 53

3.2.11 In-vivo detail activity profile of the partitioned fractions
        in rats ............................................................................. 54
i. Anti-hyperglycemic activity in normal Wistar rats .................. 54
ii. The effect of pretreatment fractions on biochemical
    parameters in normal and alloxan induced NIDDM
    Wistar rats for 42 days ....................................................... 55
iii. Collection of blood and determination of blood glucose levels 56
iv. Induction of non-insulin-dependent Diabetes Mellitus (NIDDM) .................................................. 56
v. Determination of total cholesterol (TC) ............................. 57
vi. Determination of serum high density lipoprotein cholesterol (HDL-C) .................................................. 58
vii. Determination of serum triglycerides (TG) ........................ 58
viii. Determination of low density lipoprotein cholesterol (LDL-C) 59
ix. Determination of Anti-Atherogenic index (AAI) ................. 59
x. Determination of serum insulin .................................. 60
xi. Determination of glycosylated hemoglobin (HbA1c) ........... 61
xii. Evaluation of serum creatinine and determination of renal
function of the pretreatment active fractions on rat model ....... 63

3.2.12 Evaluation of in-vitro anti-oxidant activity .................. 63
3.2.13 Statistical analysis ............................................... 65

4.0 RESULTS AND DISCUSSION

4.1. Ethno-medicinal survey .......................................... 66
4.2. Extraction of plant material ...................................... 71
4.3. Dose response studies
   4.3.1 Aqueous extracts ............................................. 72
   4.3.2 Standard anti-hyperglycemic agents ......................... 76
4.4. The effect of hypoglycemic activities of aqueous, methanol
and n-hexane extracts in normoglycemic rats ..................... 78
4.5. Bio-activity guided solvent partitioning ........................ 80
4.6. Phytochemical screening .......................................... 82
4.7. Total saponins, total alkaloids and total phenol contents ... 87
fractions in rats for 42 days ........................................... 89
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>The evaluation of Oral Glucose Tolerance activity</td>
<td>92</td>
</tr>
<tr>
<td>4.10</td>
<td>The effect of biochemical parameters in correction of hyperglycemia</td>
<td>95</td>
</tr>
<tr>
<td>4.11</td>
<td>Determination of Anti-Atherogenic index (AAI)</td>
<td>100</td>
</tr>
<tr>
<td>4.12</td>
<td>Evaluation of serum creatinine and assessment of dose dependent renal function</td>
<td>101</td>
</tr>
<tr>
<td>4.13</td>
<td>Evaluation of <em>in-vitro</em> DPPH anti-oxidant activity</td>
<td>104</td>
</tr>
<tr>
<td>4.14</td>
<td>Effect of food and water intake in rats for 42 days</td>
<td>106</td>
</tr>
<tr>
<td>5.0</td>
<td>CONCLUSIONS</td>
<td>108</td>
</tr>
<tr>
<td>6.0</td>
<td>REFERENCES</td>
<td>112</td>
</tr>
<tr>
<td>7.0</td>
<td>APPENDIX</td>
<td>131</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 4.1</td>
<td>Results of ethno pharmacological survey of medicinal plants used in the treatment of Diabetes Mellitus in Sri Lanka</td>
<td>68</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Percentage yield of water, ether and methanol soluble plant extracts</td>
<td>72</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>The dose response study of aqueous extracts of selected medicinal plants</td>
<td>75</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Comparative hypoglycemic activities of aqueous, methanol and ( n )-hexane extracts of Costus speciosus, Passiflora foetida and Osbeckia octandra in rats</td>
<td>79</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Hypoglycemic activity of solvent partitioning fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra in normal and alloxan induced NIDDM rats</td>
<td>81</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Types of phytochemicals extracted by different solvents (Hughton and Raman 1998)</td>
<td>83</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Evaluation of the cumulative activity profile of the partitioned fractions of 80% methanol extracts</td>
<td>84</td>
</tr>
<tr>
<td>Table 4.8</td>
<td>Preliminary screening of the fractions of 80% methanol extract of Costus speciosus, Passiflora foetida and Osbeckia octandra leaves</td>
<td>86</td>
</tr>
<tr>
<td>Table 4.9:</td>
<td>Total phenol, total alkaloid and total saponin contents of Costus speciosus, Passiflora foetida and Osbeckia octandra</td>
<td>87</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>Table 4.10:</td>
<td>The effect of pretreatment fractions of n-hexane, ethyl acetate and n-butanol fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on normal and alloxan-induced NIDDM rats</td>
<td>91</td>
</tr>
<tr>
<td>Table 4.11:</td>
<td>Effect of anti-hyperglycemic activity by OGTT of the fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on normal and alloxan-induced NIDDM rats</td>
<td>94</td>
</tr>
<tr>
<td>Table 4.12:</td>
<td>Effect of pretreatment active fractions on lipid profile in alloxan induced NIDDM rats</td>
<td>96</td>
</tr>
<tr>
<td>Table 4.13:</td>
<td>The effect of pretreatment of fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on biochemical parameters in alloxan-induced NIDDM Wistar rats for 42 days</td>
<td>97</td>
</tr>
<tr>
<td>Table 4.14:</td>
<td>Effect of pretreatment fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on body weight and glycosylated hemoglobin in normal and alloxan-induced NIDDM rats for 42 days</td>
<td>98</td>
</tr>
</tbody>
</table>
Table 4.15: Effect of serum creatinine and estimation of GFR on pretreatment active fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* in normal and alloxan induced NIDDM Wistar ........................................................... 102

Table 4.16: The comparison of *in-vitro* DPPH Antioxidant activity of 80% methanol extract of plants with their active fractions when compared to ascorbic acid .................................................. 104

Table 4.17: Effect of pretreatment fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* and glipizide on water intake of alloxan-induced NIDDM Wistar rats ............... 106

Table 4.18: Effect of pretreatment fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* glipizide on food intake of alloxan-induced induced NIDDM Wistar rats ............ 107
<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1: Chemical structure of sulphonylurea hypoglycemic agents</td>
<td>9</td>
</tr>
<tr>
<td>Figure 1.2: Chemical structures of biguanide hypoglycemic agents (a. Metformin and b. Phenformin)</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.3: Chemical structures of alpha-glucosidase enzyme inhibitor Acarbose</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.4: Chemical structures of Miglitol</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.5: Chemical structure of Thiazolidinediones (a. pioglitazone and b. rosiglitazone)</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.6: Chemical structures of Di-Peptidyl Peptidase-IV (DDP-IV) inhibitors (gliptins: a. Vildagliptin, b. Saxagliptin c. Sitagliptin and d. Alogliptin)</td>
<td>15</td>
</tr>
<tr>
<td>Figure 2.1 Leaves of <em>Costus speciosus</em> Linn.</td>
<td>20</td>
</tr>
<tr>
<td>Figure 2.2 Leaves of <em>Passiflora foetida</em> Linn</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2.3 Leaves of <em>Ficus racemosa</em> Linn</td>
<td>27</td>
</tr>
<tr>
<td>Figure 2.4 Leaves of <em>Osbeckia octandra</em> Linn</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.4 Leaves of <em>Averrhoa carambola</em> Linn</td>
<td>32</td>
</tr>
<tr>
<td>Figure 3.1 Preliminary extraction of plant materials for the investigation of anti-hyperglycemic activity</td>
<td>40</td>
</tr>
</tbody>
</table>
Figure 3.2: Scheme of representation of the solvent partitioning of
80% methanol extracts of *Costus speciosus*, *Passiflora foetida*
and *Osbechea octandra*.................................42

Figure 4.1: Frequency (as percentage informants) of Medicinal
Plants used in the treatment of Diabetes Mellitus in Sri Lanka...... 67

Figure 4.2: Frequency of plant families in the treatment of Diabetes
Mellitus in Sri Lanka........................................... 70

Figure 4.3: Frequency of plant parts used in the treatment of Diabetes
Mellitus in Sri Lanka........................................... 70

Figure 4.4: The dose response activity of the two standard drugs (glipizide
and metformin) on Wistar rats................................. 76

Figure 4.5: Comparison of percentage reduction in BGL vs log dose for
methanol extracts of plants and the two standard drugs
*glipizide* and *metformin* on Wistar rats......................... 77

Figure 4.6: Comparison of percentage reduction in BGL vs log dose of
fractions of plants on Wistar rats.................................. 90

Figure 4.7: Percentage change in the body weight of the pretreatment
of active fractions after 42 days when compared to the
control group..................................................... 99
Figure 4.8: Effects of fraction treatment on Anti-Atherogenic index (AAI) in normal and alloxan-diabetic rats. AAI were plotted before (0th day) and after daily oral treatment with vehicle (distilled water) and fractions for 42 days.............................100

Figure 4.9: Relation of GFR with the weight (kg)/serum creatinine (mmol/L) in rats.................................................................101

Figure 4.10: The effect of dose of active fractions vs GFR correlation of active fractions in normal and ARF induced rats......................103

Figure 4.11: Comparison of DPPH anti-oxidant effect of 80% Methanol extract of plants with their active fractions when compared to Ascorbic acid...............................................................105
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%ME:</td>
<td>80% methanol extract</td>
</tr>
<tr>
<td>AAI:</td>
<td>Anti-Atherogenic index</td>
</tr>
<tr>
<td>AE:</td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>AF:</td>
<td>Remaining aqueous fraction</td>
</tr>
<tr>
<td>ALX:</td>
<td>Alloxan monohydrate</td>
</tr>
<tr>
<td>BCG:</td>
<td>Bromo cresol green solution</td>
</tr>
<tr>
<td>BGL:</td>
<td>Blood glucose level</td>
</tr>
<tr>
<td>BF:</td>
<td>n-butanol fraction</td>
</tr>
<tr>
<td>CF:</td>
<td>Chloroform fraction</td>
</tr>
<tr>
<td>CP:</td>
<td>Corpulent rats</td>
</tr>
<tr>
<td>DM:</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>DMSO:</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPH:</td>
<td>di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium</td>
</tr>
<tr>
<td>EF:</td>
<td>Ethyl acetate fraction</td>
</tr>
<tr>
<td>ELISA:</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FBG:</td>
<td>Fasting blood glucose concentration</td>
</tr>
<tr>
<td>GAE:</td>
<td>Gallic acid equivalent</td>
</tr>
<tr>
<td>GK:</td>
<td>Goto-Kakizaki rats</td>
</tr>
<tr>
<td>HbA1c:</td>
<td>Serum glycosylated hemoglobin</td>
</tr>
<tr>
<td>HDL-C:</td>
<td>Serum high density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HF:</td>
<td>n-hexane fraction</td>
</tr>
<tr>
<td>IDDM:</td>
<td>Insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>IDF:</td>
<td>International Diabetes Federation</td>
</tr>
</tbody>
</table>
KK: Mice of the KK strain develop diabetes of polygenic origin
LDL-C: Serum low density lipoprotein cholesterol
ME: Methanol extract
NIDDM: Non-insulin-dependent diabetes mellitus
OD$_{500}$: Optical density at 500 nm
PPAR: Peroxisome proliferator activated receptor
RC: Ratio of control
RT: Ratio of test
SEM: Standard error mean
STZ: Streptozotocin
TC: Serum total cholesterol
TH$_b$: Serum total hemoglobin fraction
TZDs: Thiazolidinedione
VLDL-C: Serum very low density lipoprotein cholesterol
WHO: World health organization
ZFR: Zucker fatty rats
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisors, Dr. A. K. E. Goonathilake, Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura and Dr. G. A. Sirimal Premakumara (Doctorate Research Fellow), for his consistent supervision and dedication in guiding and following the work by devoting their golden time. And also Prof. A. M. Abeysekara, Department of Chemistry and Prof. U. G. Chandrika, Department of Biochemistry, for their constructive advice, encouragement, provision of chemicals, guidance and follow-up throughout this study. I would also like to acknowledge University of Sri Jayewardenepura and University Grant Commission for funding the project, Department of Chemistry and Department of Health Sciences for providing necessary chemicals and apparatus.

My gratitude also goes to Coordinator of the animal house for allowing me to use the animal house, and the staff of the animal house for their help in operating animal studies. I wish to thank all the staff members in Medical Research Institute, Ministry of Health, Sri Lanka, for providing me the necessary training program in animal handling.

I would also like to thank Prof. Ranil De Silva, Department of Anatomy and Prof. Kamani Samarasinghe, Dr. Inoka Uluwaduga and all the staff members of the Department of Health Sciences for their invaluable assistance during the study providing me advice.
Finally yet importantly, I would like to express my deepest gratitude to my family, especially to my mother for her encouragement and support.
ANTIDIABETIC COMPOUNDS FROM MEDICINAL PLANTS
USED IN THE INDIGENOUS SYSTEM OF MEDICINE (‘DESHIYA
CHIKITSA’) IN SRI LANKA

Malitha Aravinda Siriwardhene

ABSTRACT
The present study investigated the anti-diabetic effects of Costus speciosus, Passiflora foetida and Osbeckia octandra used in the treatment of DM in Sri Lanka. Eighty percent methanol extract (80ME) of C. speciosus, P. foetida and O. octandra leaf were evaluated for their hypoglycemic activity. Thereafer, the 80% ME extracts of plants were partitioned with organic solvents n-hexane, chloroform, ethyl acetate and n-butanol to obtain n-hexane (HF), chloroform (CF), ethyl acetate (EF) and n-butanol (BF) soluble fractions. The dose response study of the plant extracts showed, at dose of 20 mg/kg was the most effective dose. Hence, the effects of partitioned fractions of 80ME on correction of hyperglycemia were tested at a dose of 20 mg/kg in three different rat models of diabetes viz., hypoglycemic, anti-hyperglycemic and ALX-diabetic (representing the type 2 diabetic model-NIDDM) using Swiss albino Wistar rats. The effects of extracts and fractions were compared with the effect of standard drugs metformin (100 mg/kg) and glipizide (10 mg/kg).

The fractions of EF and BF of C. speciosus and EF of both P. foetida and O. octandra produced significant (p<0.05) improvement in glucose tolerance activity compared to control rats. In the long-term study, once a day administration of EF and BF of both P. foetida and O. octandra (20 mg/kg) in both normal and ALX-diabetic rats produced significant (p<0.05) antidiabetic activity. However the effect produced by P. foetida and
O. octandra fractions were lower than that of BF of C. speciosus. The study of serum biochemical parameters at a dose of 20 mg/kg showed that the 80% ME fractions of P. foetida and O. octandra have potent hypolipidemic and anti-atherogenic activities. It also improved in liver enzyme activities on both normal and ALX diabetic rats. It was observed that both C. speciosus and O. octandra fractions increased serum insulin level and lowered lipid profile significantly (p<0.05) in both normal and ALX-diabetic rats. It finally concluded that the most active partitioned fractions of these plants are BF of C. speciosus, EFs of P. foetida and O. octandra. The DPPH scavenging in-vitro anti-oxidant activities of C. speciosus (BF), O.octandra (EF) and P. foetida (EF) fractions were compared against ascorbic acid showed similar anti-oxidant activities with that of ascorbic acid. The improved renal functions along with increased in Glomerular Filtration Rate (GFR), the effect of body weight and reduced serum creatinine indicates the renal safety in chronic use of these plant fractions in the treatment of DM. The phytochemical investigation revealed that the activity profile could be due to the synergistic interaction of small molecular weight compounds present in 80% methanol extracts which may be belongs to the plant secondary metabolites viz., phenolics, alkaloids or glycoside compounds. It also proven the ethno medicinal value of C. speciosus, O.octandra and P. foetida. Further detail characterization of chemical compounds which are responsible for hypoglycemic activity of these plants may provide a pathway to discover new chemical entities in the treatment of DM.

**Key words:** Costus speciosus, Passiflora foetida, Osbechea octandra, hypoglycemia and renal function
1.0 INTRODUCTION

1.1 Diabetes Mellitus and blood glucose homeostasis

1.1.1 Diabetes Mellitus

Diabetes Mellitus (DM) is a carbohydrate metabolic disease associated with elevated blood glucose levels. The disease is characterized by either a defect in insulin action and/or sometime by the deficiency of insulin in the body. The defects in total carbohydrate metabolism affects the homeostasis disturbances of fat and protein metabolism (Barceló and Rajpathak 2001). Studies on physiology of DM has shown that by processes occurring during and after a meal, a high concentration of glucose is absorbed into blood steam. The elevated sugar in plasma activates endocrine pancreas to secrete insulin. The action of insulin on carbohydrate metabolism has been identified as uptake and storage of glucose by almost all tissue types on the body, mainly the liver. (Roussel, 1998).

The disease may also be recognized as a syndrome associate with insulin intolerance. Due to insulin deficiency and intolerance, the cells and tissues of the body are unable to absorb sufficient glucose from the blood, resulting increased glucose levels in plasma (hyperglycemia) than its normal plasma glucose values. If the hyperglycemia remains for a long period of time, it can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels causing clinical complications (Pari et al., 2004).
1.1.2 The Classification of Diabetes Mellitus

The etiology and pathogenesis in some types of diabetic phenotypes can be characterized in terms of specific reasoning but in many cases etiological and pathogenetic classification of DM is difficult (Alberti, 1992). In general, DM can be classified into two major types: insulin-dependent diabetes mellitus (IDDM, Type 1 diabetes) and non-insulin-dependent diabetes mellitus (NIDDM, Type 2 diabetes). This classification principally based on clinical symptoms and, when possible, on more specific etiologic characterization.

In IDDM, there is a necrosis of the β-cells of the pancreas, with insulin deficiency. Therefore IDDM is often associated with marked hyperglycemia with symptoms and signs of polyuria, polydipsia, and unexplained weight loss. The cause of NIDDM is often a combination of intolerance to insulin action and inadequate compensatory insulin secretion from pancreatic beta cells (Ward et al., 1984). The incidence and prevalence of diabetes varies widely throughout the world and it is based on the life style of the individual and sometimes due to atherosclerosis. However genetic and environmental factors also influence the causation of both IDDM and NIDDM (Alberti and Zimmet, 1998).
1.2 Prevalence of Diabetes Mellitus in Sri Lanka

A recent estimate indicated that 171 million people in the world were suffering from Diabetes in the year 2000 and this is projected to be 366 million by year 2030 (Ceriello and Colagiuri, 2008). Asia shows the greatest prevalence of DM (India 31.7 million and China 20.8 million) while the incidence is increasing drastically. According to a report by the International Diabetic Federation, East and North Africa have six out of the ten countries with the highest prevalence of diabetes in the world (Wild et al., 2004).

A nationwide cross-sectional study conducted between 2005 and 2006, to evaluate prevalence of DM among Sri Lankan adults showed that the provincial and ethnic distribution of diabetes closely resembled obesity (waist circumference more than the basal metabolic index-BMI) and the income level in the respective provinces and ethnic groups. When the prevalence of diabetes in Sri Lanka is concerned, one-quarter of the affected population live in the urban areas (Wild et al., 2004). It also had shown the physical activity level has an inverse relationship factor in relation of incidences of DM is concerned in Sri Lanka (Katulanda et al., 2008 and 2012). However in Sri Lanka, infectious diseases remain the most formidable enemies of health, but complicated lifestyles of the population become responsible in rising of non-communicable diseases like DM. Diabetes has become a major and growing contributor to mortality and morbidity in South Asia including Sri Lanka (Wild et al., 2004).
When considering overweight, obesity, hyperglycemia, high blood pressure and hyperlipidemia in urban populations across South Asian countries have shown high and rising rates of prevalence of risk factors for DM. Such trends also exist in rural populations but are lower in magnitude and less steep in the slope of change (Jafar et al., 2003).

1.3 Animal models in anti-diabetic evaluation

Type 2 NIDDM diabetes is presently affecting more than 100 million people worldwide and causing serious problems in the lifestyle of general public. Appropriate experimental models are essential for understanding the pathogenesis, complications, and genetic or environmental influences of the disease. It also helps to understand the risk factors of type 2 diabetes and for the testing of new chemical entities to establish as new therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. In recent years, large number of new genetically modified animals including transgenic, generalized and tissue specific modified rats and mice have been engineered and were studied experimentally (Srinivasan et al., 2005).

Animals exhibiting a syndrome of insulin resistance and type 2 diabetes, with characteristics similar to humans, comprise a wide range of species with genetic, experimental or nutritional causation. Some animals with inherent diabetes have pancreas with ‘sturdy’ beta cells. These animals are capable of maintaining
lifelong insulin secreting capacity characterized by severe hyperinsulinaemia with only mild to moderate hyperglycemia throughout the life e.g., Zucker fatty rats (ZFR), \( ob/ob \) (obese), KK mouse and corpulent (CP) rats etc. At the other end of spectrum, some species possess ‘brittle or labile’ pancreatic beta cells allowing only for transient insulin hypersecretion with short-term obesity. Subsequently, as a result of genetic predisposition and affluent nutrition or other environmental causes, it induces secretion pressure on beta cell which ultimately leads to degranulation, apoptosis and overt hyperglycemic state. At this point, the animals rapidly lose their previously accumulated adipose tissue, become ketotic and require insulin to survive. e.g., \( db/db \) (diabetic) mouse, Zucker diabetic fatty (ZDF) rats, sand rats (Psammomys obesus) and obese rhesus monkeys (Shafrir, et al., 1999). The animals with ‘brittle’ pancreas closely simulate the disease evolution from insulin resistance to progressive beta cell failure or resembled hyperglycemia as in human type 2 diabetes, than the animals with sturdy pancreas. Some of these animals with related phenotype of obesity and insulin resistance such as ZFR, ZDF rats and \( ob/ob, db/db \) and KK mice would be greatly helpful in identifying factors involved in obesity-induced diabetes (diabesity). Nevertheless, certain non-obese diabetic models are also used in the investigation of type 2 diabetes in humans that occur in the absence of obesity which allows the dissociation of confounding obesity factors such as leptin deficiency and/or leptin resistance and other associated hypothalamic factors from diabetes genes and factors viz., GK (Goto-Kakizaki) rats, akita mouse (McIntosh et al., 1999).
Chemically induced models of diabetes are common in elucidating the possible role of environmental factors involved in the endocrine pancreatic destructive processes and subsequent development of diabetes. Alloxan (ALX) was initially discovered in 1943, induced beta cell necrosis in rabbits. This compound has long been used for inducing experimental diabetes. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at alkaline pH (Bailey, 2005). ALX acts by selectively destroying the pancreatic beta islets leading to insulin deficiency producing hyperglycemia and ketosis (Rerup, 1970). ALX causes diabetes in many rodent and non-rodent animals and is most preferably used in case of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications (Battell, 1999, Kasiviswanath et al., 2005 and Sheng et al., 2005). However, guinea pig and recently musk shrew have been reported to be resistant to the action of ALX due to certain unclear mechanisms (Bell and Hye, 1983 and Ohno, 1998). Because of its low stability, relatively very shorter half-life (less than 1 min) and acidic nature of solution, intravenous or intraperitoneal routes of administration of ALX is preferred. The hypoglycemic phase may be quite severe and therefore ALX should not be given to fasted animals. The ALX treated animals exhibit severe hyperglycemia, glucosuria, hyperlipidaemia, polyphagia, polydypsia and other symptoms of uncontrolled diabetes and do also develop various complications such as neuropathy, cardiomyopathy, well-marked retinopathy and others. ALX is disadvantageous as the percentage incidence of diabetes is quite variable and is not proportionately related to increasing doses of ALX (Battell et al., 1999). Further, the incidence of
ketosis and resulting mortality is high. The reversal of hyperglycemia due to pancreatic regeneration is early and common in case of ALX treated animals. Because of these limitations, ALX is now almost replaced by Streptozotocin (STZ) for induction of diabetes in laboratory animals.

STZ is an antibiotic derived from *Streptomyces achromogenes* and structurally is a glucosamine derivative of nitrosourea. Rakieten and his associates in 1979 first demonstrated the diabetogenic property of STZ in dogs and rats. Like ALX, it causes hyperglycemia mainly by its direct cytotoxic action on the pancreatic beta cells (Ozturk *et al.*, 1996). The evidences are accumulating on the mechanisms associated with diabetogenicity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like ALX, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in STZ diabetogenicity.

There are wide variety of reports available in the literatures on doses and development of hyperglycemia with STZ since the susceptibility of animals to STZ appear to depend on age, species and even within strain (Verspohl, 2002). STZ is a preferred agent to induce experimental diabetes since it has some advantages over ALX such as, relatively longer half-life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality (Ozturk *et al.*, 1996). ALX and STZ diabetic animals are the most widely used for
screening the compounds including natural products for their insulinomimetic, insulinotropic and other hypoglycaemic or antihyperglycaemic activities (Katovitch et al., 1991, Reed and Scribner, 1999, Ho et al., 2000 and Verspohl, 2002).

1.4 Oral hypoglycemic agents

Oral hypoglycemic agents that could effectively control the abnormalities of carbohydrate, lipid, and protein metabolism that occur in patients with diabetes have been used for over half a century. There are two major structurally and functionally different oral antidiabetic drug classes, namely sulfonylureas and biguanides, which are widely used in current medicine in the treatment and the management of the anti-diabetic therapy. Glitins are a novel class of oral anti-diabetic agent that enhance and prolong the physiological actions of incretin hormones by competitively antagonizing the enzyme Di-Peptidyl Peptidase-IV (DPP-IV).

Chlorpropamide, glibenclamide, glipizide and tolbutamide are belongs to sulfonylurea class of oral hypoglycemic agents. Both pancreatic and extra pancreatic effects have been suggested to contribute to the therapeutic efficacy of sulfonylureas in the management of type 2 diabetes patients. Sulfonylureas directly stimulate insulin release from the β-cells in the islets of Langerhans, and this effect does not require the presence of glucose or other secretagogues (Gorus et al., 1988).
Figure 1.1: Chemical structure of sulfonylurea hypoglycemic agents

The chemical structure of sulfonylurea anti-hypoglycemic agents are given in the Figure 1.1. \( R_1 \) should be a \( p \)-substituted benzene. A wide variety of substituents are possible. But methyl groups on aromatic rings are usually susceptible to metabolic enzymes. They can be oxidized to carboxylic acids, which can be quickly eliminated from the body. These susceptible groups can sometimes be replaced with groups that are more stable to oxidation in order to prolong the lifetime of the drug. For example, the methyl group of tolbutamide was replaced with a chlorine atom to give chlorpropamide, which is much longer lasting. \( R_2 \) should be lipophilic with 3 – 6 carbons optimal (in case of aliphatic chain such as in tolbutamide and chlorpropamide). A single CH\(_3\) shows no activity and C\(_{12}\) shows a loss of activity relative to intermediate length chains. If an alicyclic ring is used, the number of carbons can be increased.

All sulfonylurea oral hypoglycemics inhibit the efflux of K\(^+\) (K\(^+\) channel blockers) from pancreatic β-cells via a sulfonylurea receptor which may be closely linked to an ATP-sensitive K\(^+\) channel. The inhibition of efflux of K\(^+\) leads to depolarization of the β-cell membrane and, as a consequence, voltage-dependent
Ca\textsuperscript{2+} channels on the β-cell membrane are open to permit entry of Ca\textsuperscript{2+}. The resultant increased binding of Ca\textsuperscript{2+} to calmodulin which activates kinases, associated with endocrine secretory granules. Thereby promoting the exocytosis of insulin-containing secretory granules to release insulin from the pancreas.

Among biguanides, metformin and phenformin have been commonly employed as oral anti-diabetic therapy since 1960s (Bailey, 1992). Only metformin was approved for use in the United State in early 1995. Phenformin was withdrawn in many countries during 1970s because of its association with lactic acidosis. In contrast to sulfonylureas, metformin has blood glucose reducing effect only in diabetes and it does not produce hypoglycemia in normal subjects. Metformin also exerts little or no effect on basal insulin release by the pancreas or isolated islets of nondiabetic animals (Schatz et al., 1972 and Gregorio et al., 1989).

![Chemical structures of biguanide hypoglycemic agents](image)

Figure 1.2: Chemical structures of biguanide hypoglycemic agents (a. Metformin and b. Phenformin)

The chemical structure of biguanides are given in the Figure 1.2 (a.) and (b.). Phenformin (b.) is much more lipophilic than metformin (a.). The potency of phenformin is higher than metformin. However, lipophilicity may correlate to its toxicity (lactic acidosis). It may bind more strongly to mitochondrial membranes.
and inhibits mitochondrial oxidative phosphorylation (inhibits oxygen consumption) causing a shift to anaerobic glycolysis.

The mechanism of actions of metformin include, reduction of glucose production in the liver, increase of insulin-stimulated glucose utilization in peripheral tissues (improving insulin sensitivity), and particularly skeletal muscle. It also known to be reduced intestinal glucose absorption and, apparently, increased insulin-stimulated glycogen synthesis (or reduced glycogenolysis). At the cellular level metformin is known to facilitate glucose transport across membranes by stimulating glucose transporter activity. However the precise mechanism of metformin remains to be elucidated.

The α-glucosidase inhibitors, such as acarbose was discovered in the mid-1990, has rationalized and simplified the treatment of diabetes. It is a competitive inhibitor of the major α-glucosidase enzymes in the brush border of the mucosal cell of the small intestine. It inhibits the digestion of the complex carbohydrates in the upper jejunum so that they are digested throughout the length of the small intestine. The major effect of this drug is to reduce the postprandial rise in plasma glucose (Bailey, 1992).
Acarbose (Figure 1.3) is produced by the soil bacterium *Actinoplanes* sp. Its strong inhibitory activity against α-glucosidases is widely attributed to the enhanced binding of the core aminocyclitol valienamine, whose half-chair conformation mimics the substrate distortion expected in the oxocarbonium ion transition state. In addition, the adjacent N-linked glycosidic bond prevents enzymatic hydrolysis. This amino linkage forms salt link to the acidic group of the glucosidases, which probably contributes significantly to the unusually tight binding of the inhibitor with the enzymes.

Figure 1.4: The structure of Miglitol
Miglitol is a second-generation alpha-glucosidase inhibitor derived from l-desoxynojirimycin. In contrast to acarbose, miglitol is almost completely absorbed in the small intestine.

Thiazolidinedione (TZDs) analogues (Glitazones or TZDs), a new class of antidiabetic drugs represented by ciglitazone, have been shown to be effective antihyperglycemic compounds in animal models of non-insulin dependent diabetes mellitus (NIDDM) (Fujita et al., 1983). The two analogues in this chemical series, pioglitazone and rosiglitazone (Fujiwara et al., 1988) are now available for therapeutic use (Lebovitz, 1997).

Figure 1.5: Chemical structures of Thiazolidinediones
(a. pioglitazone and b. rosiglitazone)
The thiazolidinediones (Figure 1.5) are dependent on the presence of insulin for activity. However, they do not affect insulin secretion. The thiazolidinediones are highly selective and potent agonists for the peroxisome proliferator activated receptor gamma (PPARγ) that regulates the transcription of a number of insulin responsive genes. PPARγ receptors can be found in key target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. For example, stimulation of these receptors may result in increased production of GLUT1 and GLUT4 receptors. Additionally, PPARγ responsive genes also play a role in the regulation of fatty acid metabolism. Unlike oral sulfonylureas, rosiglitazone enhances tissue sensitivity to insulin rather than stimulates insulin secretion. Also, based on this mechanism, it may take several weeks for these drugs to fully express their activity (and thus to assess their potential).
A number of DDP-IV inhibitors (gliptins) are a new class of oral antihypoglycemic medication (Figure 1.6: a. Vildagliptin, b. Saxagliptin c. Sitagliptin and d. Alogliptin) (Noriyasu, 2011) that prolong the physiological actions of Glucagon-like peptide 1 (GLP-1). Vildagliptin and sitagliptin newest gliptins that are under investigation for the treatment of type 2 diabetes in humans (Pederson 1998 and Sudre 2002). The recent clinical trial data looking at their use as monotherapy and combination therapy with conventional oral hypoglycaemics. Short term clinical trials show that gliptins cause a modest reduction in glycated haemoglobin when used as monotherapy or combination therapy. They appear to be more potent when baseline glycated haemoglobin is higher (Ahren, 2000). They appear to be well-tolerated, and are taken orally once daily. They may be useful in treating obese patients with type 2 diabetes, in combination with metformin, or a thiazolidinediones, or both. In general, gliptins appear to be safe
and well tolerated, with few reported side-effects they are neither cause weight gain nor loss and unlikely to cause hypoglycemia (Pospisilik 2002).

1.5 Phytomedicines in Diabetes Mellitus

Plant secondary metabolites are frequently associated with the bioactivity of plants which have been investigated by previous investigators. They usually combine with plant taxons. The isolation of chemical compounds or fractions with optimum therapeutic activity with no toxicity is the intention of modern research on phytochemistry and ethanopharmacology. Traditional herbal medicine has been used since ancient time in many parts of the word. DM is one of the disease that the extensive use of medicinal plants were involved especially in Asian region. There are many side effects of insulin therapy and other oral hypoglycemic agents necessitate the use of more effective and safer antidiabetic drugs. For example, long-term use of Metformin causes diarrhea, nausea, weakness, indigestion, abdominal discomfort and headache while sulphonylurea causes weight gain and hypoglycemia (Bolen et al., 2007). Plant based drug are considered to be less toxic and free from side effects than synthetic therapeutic compounds. Hence, they play an important role as alternative medicine. Phytomedicines are mainly whole, fragmented or cut, plants parts of plants, algae, fungi or lichen in an unprocessed state. They are used in a dried form, but sometimes fresh areal plant parts also used. Herbal drugs are defined by their botanical (scientific) binomial. The active principles present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing.
and reversing complications due to insulin resistance (Welihinda et al., 1982). Anti-diabetic plants also possess strong antioxidant/free-radical scavenging properties.

Medicinal and treatment methods in Sri Lankan traditional system of medicines are quite similar to that of Indian ayurvedic methods. However Sri Lankan traditional system of medicine has some unique and even techniques, which are inheriting to Sri Lanka. It was believed that these techniques have been developed, practiced and even improvements added on from time to time, since the country was inhabited. The traditional physicians of Sri Lanka use a wide variety of herbs to prepare various medicines for different ailments. Some of the valuable medicinal plants used therein are considered as weeds by agriculturists, from their focal point of view. Exploitation of such herbs is also beneficial as they conserve soil by serving as a live ground cover in crop lands. Doing away with chemical weed control also caused to minimize possible hazards of herbicides on humans, animals and environments.

Petroleum derived raw materials are used in chemical industry and they are the precursor materials for many of synthesized medicines in modern day. The limitation of petroleum causes difficulties in supplying the demand to the bulk synthesis of medicine. Plant derived medicine is therefore an utmost traditional way of finding though it has been under estimated in modern medicine are now becoming serious consideration among modern scientists and phytochemists. Quite a lot of medicinal plants have been chemically investigated for their medical
potential (Farnsworth, 1966 and Farnsworth and Soejarto, 1991). However fairly a few number of them have been investigated and scientifically proven for their bioactivity.

Medicinal plants have been used to cure major chronic ailments as a source of medicine. Investigators have reported that about 80-85% of population in both developing and some developed countries rely on traditional medicine (Tomlinson and Akerele, 1998, Elujoba et al., 2005 and Ignacimuthu et al., 2006). As a consequence, there is an increasing trend, worldwide, to integrate traditional medicine with health care in the treatment of chronic diseases including DM.

Identifying bioactive phytochemicals can offer solutions to modern day to explore the scientific basis of new therapeutic compounds. Sri Lanka has long history of medical tradition and the traditional learning of plant remedies for many diseases including diabetes mellitus. Sri Lanka is a tropical island with monsoons, located between 50-100 N and 790-830 E with an average temperature between 13°C and 31°C and receive 1300-3500 mm of rain each year. A reasonable amount of Sri Lankan medicinal plants are identified and scientifically evaluated for their activities in the treatment of DM. These plants are used in the form of decoctions or khyme (a thick gruel prepared by boiling a handful red rice and selected plants with water and consumed before meals. (Ediriweera and Ratnasooriya, 2009).
LITERATURE REVIEWS

2.1 Anti-diabetic ethno-medicine in Sri Lanka

In Sri Lanka, especially in the rural areas, ayurvedic medicine had been prescribed for diabetes since ancient times. An approximately 40 plant species have been used in such treatment (King et al., 1993). Majority of these plant species have been subjected to scientific investigation (Attygalle et al., 1917). The hypoglycaemic activity of a few plant species such as *Salacia reticulata* (Karunanayake et al., 1984 and Yoshikawa et al., 1998), *Momordica charantia*, *Aegle marmelos* (Karunanayake et al., 1984), *Ficus benghalensis* (Fernando et al., 1987, Seetha et al., 1994 and Atta-Ur-Rahaman et al., 1989), *Bambusa vulgaris* (Fernando et al., 1990), *Momordica dioica* (Fernanopulle et al., 1994) has already been established.

Extracts of plants such as, *Salacia reticulata*, *Aegle mameleos*, *Momordica charantia* (Karunanayake et al., 1984) and *Ficus benghalensis* (Fernando et al., 1987) have shown hypoglycemic activity on normal fasting rats over a 4 h study period. Most of the previous works on hypoglycaemic effect have performed using glucose oxidase method to determine the blood glucose level (Karunanayake et al., 1984, Fernando et al., 1984, 1987, 1990, and 1994, Fernanopulle et al., 1994 and Canagaratna and Sivagowry, 1998).
2.2 Costus speciosus Linn

The scientific name of the plant is Costus speciosus (Koen.) Sm. It is commonly known as "crepe ginger" or "spiral-flag" in English, "thebu" in Sinhala and "kostum" in Tamil. The plant is widely distributed in Asia and tropical countries like Sri Lanka, India, Nepal, Pakistan, Taiwan, Malaysia and China. In Sri Lanka, it occurs mostly in wet zone covering Western, Southern and Sabaragamuwa provinces.

C. speciosus consists of two varieties viz., var. nepalensis found only in Nepal and India and var. argyrophyllus having a wide distribution in other countries. A taxonomic rank controversy regarding C. speciosus was pointed out by Gideon, 1991. The taxonomic position of the natural group comprising C. speciosus (Dimero), C. speciosus (Mono) and C. speciosus (Tapeinochilos) has been
reviewed by Gideon 1991. An explanation has also been given for the preferred taxonomic rank for the *C. speciosus* group.

The botanical description of the plant was given by Gamble, 1987, Kirtikar and Basu, 1975 and 1987 and Sivarajan and Balachandran, 1994. The plant is a succulent herb with long leafy spirally twisted stems, 2-3m in height and horizontal rhizomes. Leaves are simple, spirally arranged, oblanceolate or oblong, glabrous above, silky pubescent beneath with broad leaf sheaths. Flowers are white, large, fragrant, arranged in dense terminal spikes and bracts are bright red. The single stamen present is perfect, lip large with incurved margins. Fruits are globose or ovoid capsules with obovoid or sub-globose seeds (Warrier *et al.*, 1994).

*C. speciosus* is valued very much for its diosgenin content in its rhizome. It is widely used as starting material in the commercial production of steroidal hormones. The rhizomes are useful in vitiated conditions of ayurveda, burning sensation, flatulence, constipation, helminthiasis, leprosy, skin diseases, fever, hiccough, asthma, bronchitis, inflammation and anemia. It is also used to make sexual hormones and contraceptives (Warrier *et al.*, 1994). The plant is often cultivated as ornamental herb (Chopra *et al.*, 1980). One of the constituents of *C. speciosus* extracts is useful in rheumatism. Juice of stem bark taken in burning sensation on urination. Juice of boiled plant is used in earache (Asolkar *et al.*, 1992).
The previous investigators have showed the tubers and roots of C. speciosus contain diosgenin, 5 ɑ-stigmast-9 (11)-en-3-β-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinones. It also reported that the plant contains saponins together with many new aliphatic esters and acids in its rhizomes, seeds and roots. Seeds, in addition, are reported to be contained α-tocopherol (Husain et al., 1992).

Ethnomedical uses of C. speciosus indicated that saponins from seeds are hypotensive and spasmolytic. Whereas, rhizomes possess antifertility, anticholinesterase, antiinflammatory, stimulant, depurative and anthelmintic activities (Husain et al., 1992). Saponins (Diosgenin and tigogenin) were known to produce estrogen like activity in albino rats similar to stilbestrol. Essential oil from rhizome are reported to be antimicrobial (Asolkar, 1992). The previous investigators had investigated the activities of alkaloidal fraction from rhizome. It showed papaverine like smooth muscle relaxant, antispasmodic, cardiotonic, hydrochlororetic, diuretic and CNS depressant activities in laboratory animals. Fairly a very few reports were found the activities and phytochemistry of C. speciosus leaves. Where, β-amyrin stearate, β-amyrin and lupeol palmitates from leaves were isolated. However the pharmacological activities are unclear. It has been reported that the rhizomes contained largest amount of sapogenin (2.7%) (Rastogi and Mehrotra, 1991). Differential diosgenin accumulation in C. speciosus was studied by and found out that the diosgenin content of field grown C. speciosus plants was high in the rhizomes and very low in the other parts of the plant (Indrayanto et al., 1994). Therefore from the various types of tissue
cultures which have initiated from the same cluster of plants, only shoot leaf cultures and shoots of the plantlet cultures contained diosgenin. The compound was not detected in callus cultures, root cultures and roots of the plantlet cultures. The antifungal activity was tested at different concentrations of steroidal saponins and sapogenins from *C. speciosus* by Singh *et al.*, (1992). The Saponins isolated from *C. speciosus* was found to be highly effective against conidial germination of fungus *viz.*, *Botrytis cineria* and *Alternaria sp.* investigated by the same author. Pharmacological studies conducted by Bhattacharya *et al.*, (1973) showed that the rhizomes of *C. speciosus* possess cardiotonic, hydrochloretic, diuretic and CNS depressant activity.
2.3 *Passiflora foetida* Linn

*Passiflora foetida* (Linn) belongs to *Passifloraceae* family is a herbaceous climber, native of tropical America and found wild in several parts of India and Sri Lanka. It is commonly called as “Stinking passion flower” in English, “Pada wel” or “Wal wel dodam” in Sinhala and “Mupparisavalli” or “Siruppunaikkalli” in Tamil (Narayan *et al*., 2003, Khare, 2007 and Norman, 2001). *P. foetida* is a woody, annual or perennial vine, 1.5 to 6 m long; stem, cylindrical, densely hairy; tendrils arise next to leaves on the shaded side of the stem; leaves heart-shaped to three lobed, alternate, and arranged helically, with long-stalked glands and long fine hairs on margins, producing a disagreeable smell when crushed. The flowers are white to lilac in colour and bisexual. They flower all year round, opening in the morning and closing before noon. The green to orange or red fruits are enclosed in lacy bracts. The traditional medicinal practitioners use the plant *P.*
foetida, commonly known as granadilla to treat various neurological disorders. Therefore according to traditional practice, the decoction of leaf is used for nervous disorders (Vedavathy, 1997).

The genus Passiflora may be suitable for the screening of bioactive molecules, since ethnobotanical use, chemotaxonomic information, and observation of the interaction of the plants with their environment have been suggested as selection criteria for potential sources of natural molecules of pharmacological relevance (Rates, 2001).

The whole plant is used in the treatment of insomnia and anxiety. The decoction of fruit is used for asthma and biliousness. Decoction of leaves and roots is emmenagogue and also used in hysteria. The plant is used for curing itches. The major components present in the plant are maltol, phytosterols, cyanogenic glycoside, flavonoids and their glycosides (Manuchair, 2007).

A very few reports were found on phytochemistry and ethnopharmacology of P. foetida. However the genus Passiflora had been investigated extensively. Phytochemical investigation of P. incarnate and P. edulis and the occasional analysis on other species revealed that the members of this genus contain alkaloids, phenols, cyanogenic compounds and glycosyl flavonoids (Dhawan and Kumar, 2002 and Dhawan et al., 2004). The pharmacological activity of some of these compounds, such as chrysin found in P. edulis (Medina et al., 1990) and maltol, ethyl maltol, flavonoids and harman alkaloids in P. incarnata had been
reported (Aoyagi et al., 1974 and Soulimani et al., 1997). *P. Incarnata* has aromatase properties due to the presence of two flavonoid compounds: chrysin and benzoflavone moiety, the latter being more potent antioxidant (Dhawan et al., 2002). Many species have been found to contain β-carboline group of alkaloids.

The majority of the active components in this plant are C-glycosyl flavones based on apigenin and luteolin, while alkaloids were found in trace amounts (Hiremath et al., 2000, Rehwald et al., 1994 and Raffaelli et al., 1997). Several flavonoids, chrysinand apigenin, have been isolated from *P. Incamata* (Zanoli et al., 2000) along with orientin, isoorientin, vitexin and isovhexin (Soulimani et al., 1997). The largest accumulations of *P. Incarnata* flavonoids were found in the leaves between the pre-flowering and flowering stages of the plant (Menghini and Mancini, 1988).
2.4 *Ficus racemosa* Linn

It is popularly known as “Country fig or Fig leaves” in English, “aththikka” in Sinhala and “Atti” in Tamil. Several species belonging to the genera of *Ficus* were reported to contain furanocoumarins which is an important plant phototoxins (Swain and Downum, 1990). *F. racemosa* Linn (*Moraceae* family) is an evergreen, moderate to large sized spreading, lactiferous, deciduous tree, without much prominent aerial roots. The plant grows all over India and Sri Lanka in many forests and hills. It is frequently found around the water streams and is also cultivated. The tree is medium tall with quite rich green foliage and growing up to 10-16 m in height. The rich green foliage provides a good shade. The bark is reddish grey and often cracked. The leaves are dark green, 7.5-10 cm long, ovate or elliptic. The fruit receptacles are 2-5 cm in diameter, pyriform, in large clusters,
arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening. The seeds tiny, innumerable, grain like, the outer surface of the bark of the seed consists of easily removable translucent flakes grayish to rusty brown, uniformly hard and non-brittle (Mandal et al., 2000).

The antioxidant properties of *F. racemosa* have been demonstrated by previous investigators. It showed the antioxidants isolated from the plant can protect lipoproteins in plasma from oxidation and produce a significant increase in plasma antioxidant capacity (Duenas et al., 2008). All parts of this plant (leaves, fruits, bark, latex, and sap of the root) are medicinally important in the traditional system of medicine in India. The powdered leaves mixed with honey is given in bilious infections (Kirtikar and Basu, 1975). Fruits are a good remedy for visceral obstruction and also useful in regulating diarrhea and constipation (Vihari, 1995).

The astringent nature of the bark has been employed as a mouth wash in spongy gum and also internally in dysentery, menorrhagia and haemoptysis (Chopra et al., 1956). The bark is reported for it’s an antiseptic, antipyretic and vermicidal activity. The decoction of bark is used in the treatment of various skin diseases, ulcers and diabetes. It is also used as a poultice in inflammatory swellings or to treat boils and regarded to be effective in the treatment of piles, dysentry, asthma, gonorrhea, gleet, menorrhagia, leucorrhea, hemoptysis and urinary diseases (Nadkarni et al., 1976).
Apart from the usage in traditional medicine, scientific studies indicate *F. racemosa* to possess various biological effects such as hepatoprotective (Mandal et al., 2000), chemo preventive (Khan and Sultana, 2005), anti-inflammatory (Mandal et al., 2000), antipyretic (Rao et al., 2002), antitussive and antidiuretic (Ratnasooriya et al., 2003). Rao et al. (2002 and 2003) had investigated the antidiabetic activity of the bark powder. The bark has also been evaluated for cytotoxic effects using 1BR3, Hep G2, HL-60 cell lines and found to be safe and less toxic when compared to aspirin, a commonly consumed anti-inflammatory drug (Li et al., 2004). However antidiabetic activity of *F. racemosa* leaves were not investigated.

The phytochemistry of the plant has been reported by previous investigators. The stem bark of *F. racemosa* reported to be contains tannin, wax, saponin guananol acetate, $\beta$-sitosterol, leucocyanidin-3-O-$\beta$-D-glucopyranoside, leucopelargoidin-3-O-$\beta$-D-glucopyranoside and leucopelargoidin-3-O-$\alpha$-L-rhamnopyranoside (Deva raj, 2008). Lupeol, ceryl behenate, lupeol acetate, $\alpha$-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin from trunk bark, lupeol, $\beta$-sitosterol and stigmasterol were also isolated. Fruit contains guananol, hentriacontane, $\beta$-sitosterol, guananolacetate, glucose, tiglic acid, and esters of taraxasterol, lupeolacetate, friedelin, higher hydrocarbons and other phytosterol (Suresh et al., 1979). A new tetra-triterpene guananol acetate which is characterized as 13$\alpha$, 14$\beta$, 17-$\beta$-H, 20, $\alpha$-H-lanosta-8, 22-diene-3$\beta$-acetate and racemosic acid were isolated from the leaves. An unusual thermos-stable aspartic
protease was also isolated from the latex of the plant. The stem bark and fruit showed the presence of glauanol acetate (Deva Raj, 2008).
Osbeckia octandra is a plant species belonging to the family Melastomaceae, which is found in Sri Lanka (Dassanayake, 1995). It is referred to as "Heenbovitiya" in Sinhala and "Eight Stamen" in English. It is a perennial shrub and it has attractive pale pink or pinkish purple flowers can be seen in grasslands. Leaves of this plant are used in Sri Lankan traditional ayurvedic medicine as a treatment for liver related deceases. The leaves of this plant have a sweet and acidic taste.

*O. octandra* is a branched shrublet, growing up to 2 m tall. Branches are sparsely velvety with prostrate or spreading hairs. Leaves are elliptic, 1.5-6 cm long, 0.5-2 cm wide, rounded to point at the base, with a pointed tip. The leaves contain...
three nerves, generally hairless or sparsely velvety, carried on a 1-5 mm long stalk. Flowers are few, up to 19 in number per tree, borne in loose clusters. Sepals are triangular, 2-4 mm long, bristled at the tip. Petals are 1.5-1.9 cm long, pink to mauve or purple. Stamens are 8 cm, with large, narrowly ovate and twisted anthers of 6-8.5 mm long. *O. octandra* is endemic to Sri Lanka, however the plant is reported to be growing in wild in southern states of India.

Reports indicated that ayurvedic and traditional medical practitioners in Sri Lanka use mature leaves of the *O. octandra* for its hepatoprotective properties. The juice extracted from its leaves is reported as an effective treatment for liver damage caused by paracetamol (Acetaminophen) poisoning. (Attygalle *et al.*, 1917). The plant is used in traditional medicine for the mitigation of jaundice related to liver disease. Although many investigations have been carried out to assess the effect on liver diseases of *O. octandra*, (Jayaweera *et al.*, 1982, Jayathilaka *et al.*, 1989 and Thabrew *et al.*, 1987 and 1995), a very little information is available on the relative potencies of *O. octandra* as a plant with anti-diabetic potential. However a study of antiglycation and antioxidant activities of aqueous leaf extract of *O. octandra* have been demonstrated confirming the long history of the use of the plant in traditional medicine in Sri Lanka (Jayathilaka *et al.*, 1989). However *Osbeckia* species was known to have antioxidant compounds such as flavonoids, hydrolyzable tannins, osbeckic acid and furancarboxylic acids (Su *et al.*, 1987 and 1988). A proper scientific investigation of the activity profile of *O. octandra* is yet to be discovered.
2.6 *Averrhoa carambola* Linn

![Figure 2.5: Leaves of *Averrhoa carambola* Linn.](image)

*Averrhoa carambola Linn. (Averrhoaceae family)* is known to have originated in Sri Lanka and the Moluccas. It has been cultivated in Southeast Asia and Malaysia for hundreds of years (Morton, 1987). The plant is a small, multistemmed, slow growing evergreen tree with a short trunk or a shrub, grown up to 5-7 m of height or rarely, 10 m high. It has a bushy shape with many branches producing a broad, rounded crown. At the base, the trunk reaches a diameter of 15 cm (Morton, 1987 and Kapoor, 1990). Purple to bright purple colored flowers are produced in the axils of the leaves. The flowers are arranged in small clusters and each cluster is attached to the tree with red stalks. The flowers are small, about 6 mm wide,
pedicellate with 5 petals (having curve ends) and sepals (Nicholson, 1969 and Perry, 1980).

It was reported the isolation and identification of a few compounds such as β-sitosterol, lupeol and 1, 5-dihydroxy-6, 7-dimethoxy-2- methyl-anthraquinone 3-O-β-glucopyranoside. (Huang, 2009 and Moresco, 2012). However fifteen compounds, which included six chiral lignans and nine phenolic glycosides, were separated from the butanol fraction of A. carambola root and identified. (Huang 2009). Few of them are namely 3, 4, 5-trimethoxyphenol-1-O-β-D-glucopyranoside, (-)-lyoniresinol 3α-O-β-D-glucopyranoside (-)-lyoniresinol 3α-O-β-D-glucopyranoside 3, 4, 5-trimethoxyphenyl and 1-O-β-apiofuranosyl (Gunasegaran, 1992, Araho, 1992 and Moresco, 2012).

This tree is also known as the star fruit tree in English, Kamaranga in Sinhala and Thambaratham/Tamarattai in Tamil. It is commonly used to treat headaches, vomiting, coughing and hangovers (Rangari, 2008). Furthermore, it is used as an appetite stimulant, a diuretic, and as an antidiarrheal and febrifugal agent. A. carambola has been used in the treatment of eczemas (Evance, 1997). In addition, the extract obtained through decocting the leaves of A. carambola has been used in the treatment of diabetes (Rangari, 2008).
2.6 Aims and objectives of the thesis

2.6.1 Overall objective

To explore the scientific basis of the bioactive fraction/compounds from selected medicinal plants used in the treatment of diabetes mellitus by ayurvedic and traditional medicinal practitioners in Sri Lanka on biochemical and enzymatic parameters of different rat models, *in-vitro* bioassays and insulin index for correction of hyperglycemia.

2.6.2 Specific objectives

i. To carry out a field survey and literature survey to identify medicinal plants used in the treatment of diabetes mellitus by ayurvedic and traditional medicinal practitioners in Sri Lanka.

ii. To determine the hypoglycemic activity of extracts/fractions from selected medicinal plants using biochemical and enzymatic parameters of different rat models and/or *in-vitro* bioassays for correction of hyperglycemia.

iii. To isolate and identify active compounds from active fractions by activity directed fractionation.

iv. Detailed study of the activity profile of active fraction and compounds using biochemical and enzymatic parameters of different rat models, *in-vitro* bioassays and insulin index for correction of hyperglycemia.
3.0 MATERIALS AND METHODS

3.1 Materials

3.11 Chemicals and reagents

**BDH Laboratory Supplies (India)**

Sodium citrate, sodium chloride, methanol, n-hexane, ethyl acetate, n-butanol, acetone, Conc. hydrochloric acid and Conc. sulphuric acid, basic bismuth nitrate, glacial acetic acid, chloroform, calcium hydroxide, acetic anhydride, ferric chloride, ferric sulphate, lead acetate, sodium hydroxide, formaldehyde, anesthetic ether, potassium hydroxide, benzene, ammonia solution, sodium bicarbonate, bromocresol green, sodium phosphate, citric acid, anhydrous sodium sulphate and \( p \)-anisaldehyde

**Sigma-Aldrich (St Louis, MO, USA)**

Alloxan monohydrate, silica, gallic acid (standard), digoxin (standard), metformin (standard), glipizide (standard), 2,2-diphenyl-1-picrylhydrazyl (DPPH), atropine sulphate (standard) and ascorbic acid (standard).
3.1.2 Kits

**Boehringer Mannheim (GmbH, Mannheim, Germany)**

Cholesterol (TG) reagent; Peridochrom® Triglyceride (TC) reagent

HDL Cholesterol kit (CHOD-PAP method)

3.1.3 Facilities

Buchi rotary evaporator R-144 (Buchi Labortechnik AG, Switzerland)

Buchi water bath B-480 (Buchi Labortechnik AG, Switzerland)

Chemical hood

-20°C Freezer (Forma Scientific)

-20°C and 4°C LG Refrigerator (South Korea)

Balance (Shimatzu™ VS 3000 series, Japan)

Analitica™ J-251 Centrifuge (India)

Shimatzu™ GS 5000 series UV-visible spectrophotometer (Japan)

3.1.4 Animals

Male Wistar rats, aged 10 weeks (150-220 g), were obtained from the laboratory animal center, Medical Research Institute, Ministry of Health, Colombo, Sri Lanka.
The rats were housed two per cage in a room with a 12-h light and 12-h dark and an ambient temperature of 25-28°C in an animal room at Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka, with lighting from 0600 to 1800 h and maintained on standard pelleted diet, with water ad libitum.

3.2 Method

3.2.1 Survey on antidiabetic medicinal plants used in Sri Lanka

As approach 31 auyrvedic medical practitioners and traditional healers were interviewed with organized semi-structured interviews. Each of them was met two times, at different moments, to answer the same questions. The plants used for the treatment of diabetes mentioned in these studies were selected by searching for terms such as “diabetes” and “lower the blood sugar”. This helped to check the information which had already reported by other investigators. During this ethno medicinal investigation, it was collected information related to plants used to treat diabetes, the different parts of the plant used as ‘the medicamentous drugs’ and the modes of preparation and administration of the medicamentous receipts were collected. From the collected samples and specimens kept at the herbarium the plants were identified, by their scientific name and determined their botanical characteristics. For the information concerning the plant parts used in treatment of diabetes has been standardized. Terms related to liquid preparations such as infusion and decoction have been standardized as “aqueous extract”, since these methods just differ in the extraction time and the temperature reached were
categorized as aqueous extract. The term “dry extract” was used to describe semisolid medicamentous drug prepared from the aqueous extracts.

The collected data was represented by graphical representation made to facilitate short listing of plants (Table 4.2 and Figure 4.2).

3.2.2 Plant material

Fresh leaves of the species of Costus speciosus, Passiflora, foetida, Osbeckia octandra, Averrhoa carambola, and Ficus racemosa were collected from Piliyandala and Baduraliya, Colombo district, Western Province of Sri Lanka. The taxonomic identification of all plants were done by a taxonomist from Peradeniya Royal Botanical Gardens, Peradeniya, Sri Lanka.

3.2.3 Extraction of plant material

The leaves were air dried for 48 hours and ground using a mechanical grinder to a coarse powder before extraction. The extracts for preliminary investigation were prepared by reflux extraction method. A 200 g of the powdered plant material of respective plants were refluxed using 1000 ml of distilled water, 80 % methanol and n-hexane, separately for one hour. All extracts were filtered (Watman filter paper no. 4) and dried under vacuum (Büchi rotary evaporator, West Germany) at 40°C and the yields were recorded. The percentage yields were reported and the dried extracts were labeled as “aqueous” (AE), “80% methanol (80ME) and “n-
hexane” (HE) respectively and preserved under nitrogen in a refrigerator at 4°C till it use in the subsequent experiments. The extraction scheme is represented in Figure 3.1.

![Extraction Scheme](attachment:image)

**Figure 3.1:** Preliminary extraction of plant materials for the investigation of anti-hyperglycemic activity

### 3.2.4 Bioactivity guided solvent partitioning of 80% methanol extracts

Dried extract of 80% ME of *C. speciosus, P. foetida* and *O. octandra* leaves (100 g each) were dissolved in 1000 ml of distilled water. The solvents for partitioning were chosen on the ground of their polarity, boiling points as well as their ability to evaporate, and represented a polarity range from non-polar to polar. The solvents selected were: *n*-hexane, chloroform and ethyl acetate. 80% ME extract of the respective plant were suspended in distilled water and partitioned successively with (5 X 250 ml) with each solvents. (Figure 3.2). The resultant fractions of *n*-hexane, chloroform and ethyl acetate were labelled as HF, CF and EF respectively. The remaining aqueous fraction (AF) was successively partitioned with *n*-butanol to obtain *n*-butanol fraction (BF). All the solvents used
were Analar-pure grade (BDH India) and rectified for their boiling points before extraction. The resultant fractions of \( n \)-hexane (HF), chloroform (CF), ethyl acetate (EF) and \( n \)-butanol (BF) fractions were dried under vacuum at 40\(^0\) C. The yield was determined, placed in a desiccator overnight and preserved under nitrogen filled tightly sealed dark coloured glass containers in a refrigerator at 4\(^0\) C.
One hundred gram of 80% methanol 
Dissolved in 1000 ml of distilled 
water filtered and extracted with n-
hexane

$n$-hexane fraction (HF) 

Aqueous layer

Extracted with CHCl$_3$

CHCl$_3$ fraction (CF)

Aqueous layer

Extracted with EtOAc

EtOAc fraction (EF)

Aqueous layer

Extracted with $n$-butanol

$n$-butanol fraction (BF)

Aqueous residue

Figure 3.2: Scheme of representation of solvent partitioning of 80% methanol extracts of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra*
3.2.5 Preliminary phytochemical screening of 80% methanol extracts

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures (Mukherjee, 1986 and Farnsworth, 1996).

i. Test for alkaloids

Preliminary test:
A 100 mg of an alcoholic extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendorff’s and Mayer’s reagents. The treated solutions were observed for any precipitation.

Confirmatory test:
Five grams of the alcoholic extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated \textit{in-vacuo} to about 5 ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared
Dragendorff's spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidence for presence of alkaloids.

ii. Test for steroidal compounds

Salkowski's test:
To 0.5 g of the alcoholic extract was dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid (i.e. the aglycone portion of the glycoside).

Lieberman's test:
To 0.5 g of the alcoholic extract was dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A colour change from purple to blue to green indicated the presence of a steroid nucleus i.e. aglycone portion of the cardiac glycosides.
iii. Test for phenolic compounds

Test A:
To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1% ferric chloride and 1 ml of potassium ferrocyanide was added to detect phenolic compounds. Formation of bluish-green color was taken as positive.

Test B:
The dried alcoholic extract (100 mg) was dissolved in water. A few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

iv. Test for flavonoids

Test for free flavonoids:
Five milliliters of ethyl acetate was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle and inspected for the production of yellow colour in the organic layer which is taken as positive for free flavonoids.
**Lead acetate test:**

To a solution of 0.5 g of the extract in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

**Reaction with sodium hydroxide:**

Dilute sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The mixture was inspected for the production of yellow color which considered as positive test for flavonoids.

v. **Test for saponins**

**Froth test:**

To 0.5 g of the alcoholic extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minute period of time. If a “honey comb” froth above the surface of liquid persists after 30 min. the sample is suspected to contain saponins.
vi. Test for tannins

Ferric chloride test:
A portion of the alcoholic extract was dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

Formaldehyde test:
To a solution of about 0.5 g of the extract in 5 ml water, 3 drops of formaldehyde and 6 drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 minute and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.

Test for Phlobatannins:
Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as an evidence for the presence of phlobatannins.
vii. **Test for Anthraquinones**

**Test for free anthraquinones (Borntrager’s test)**

The aqueous-alcoholic extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

**Test for O-anthraquinone glycosides (Modified Borntrager’s test)**

For combined anthraquinones, 5 g of the plant extract was boiled with 10 ml 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extract.
3.2.6 Quantitative determination of total phenols

The total content of phenols in the crude chloroform and ethyl acetate extracts was determined using a modified Folin-Ciocalteu colorimetric method with gallic acid as a standard. The extract solution in DMSO (700 μL) was transferred to a 10 ml volumetric flask, the Folin-Ciocalteu reagent (400 μL) was added and after 3 min, each flask was made up to the mark with sodium carbonate (Na₂CO₃) solution (75 g/L). After 2 hours, the suspension was centrifuged (5000 rpm, 5 min) and the absorbance of the solution was measured at 760 nm. The total phenolic content was expressed as a gallic acid equivalent (GAE) in g/100 g of dry extract. Data are reported as mean ± SD for at least three replicates.

3.2.7 Spectrophotometric determination of total alkaloids

Preparation of solutions:
A 1x10⁻⁴ diluted bromocresol green solution (BCG) was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na₂HPO₄ in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1 mg pure atropine (Sigma Chemical, USA) in 10 ml distilled water.
Preparation of standard curve

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separator funnels. Then, add 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

3.2.8 Spectrophotometric Determination of Saponins

Sample Preparation

Twenty g of silica was soaked in methanol overnight and packed in a glass column of 15 mm diameter with methanol. The silica column was washed with 100 ml methanol and 200 ml water successively. Plant extracts (200 mg), was charged to the column with a small amount of water, and washed with 100 ml water and 100 ml 40% methanol successively. Saponin was eluted with 100 ml 95% methanol solution. The solvent in the saponin fraction was removed under reduced pressure. The residue was dissolved with methanol and made up to 20 ml for the hydrolysis step. Hydrolysis Five to 20 ml of the methanol solution of saponin was placed in a short neck flask. Methanol was removed under reduced pressure. Ten ml of 2 mol/L HCl and 10 ml ethanol were added to the residue and hydrolyzed for 3 h at 90°C, cooled, and extracted twice with 80 ml diethyl ether. The saponin fraction in the ether layer was washed with 20 ml water and dehydrated with 20 g sodium
sulfate anhydride. The ether was removed under reduced pressure and the residue which contained sapogenin was dissolved with ethyl acetate and made up to 10 ml for spectrophotometry.

**Spectrophotometric Determination**
The following colour developing reagent solutions were prepared: (A) 0.5 ml of \( p \)-anisaldehyde and 99.5 ml ethyl acetate, and (B) 50 ml concentrated sulfuric acid and 50 ml ethyl acetate. The ethyl acetate solution containing sapogenin was diluted with ethyl acetate to contain 2.5 to 10 mg/ml sapogenins. Two ml diluted sapogenin solution was placed in a 10 ml test tube. One ml each of reagent solutions (A) and (B) were added and the test tube sealed with a glass stopper. After stirring, the test tube was placed in a water bath maintained at 60 °C for 10 min to develop color, then allowed to cool for 10 min in a water bath maintained at room temperature. Because the boiling point of ethyl acetate is 70 °C, the water bath temperature should be controlled accurately. The absorbance of the color-developed solution was measured.

Ethyl acetate was used as a control for the measurement of absorbance. As a reagent blank, 2 ml ethyl acetate was placed in a test tube and assayed in a similar way as the sapogenin solution. Solutions containing 2–40 mg digoxin (standard) in 2ml ethyl acetate were used to obtain a calibration curve.
3.2.9 Preliminary hypoglycemic activity of aqueous extracts

Male albino Wistar rats (150-220 g) were grouped into six groups (Group I – VI) for the evaluation of preliminary hypoglycemic activity. Groups II-VI, further subdivided into five groups (n = 6). An oral glucose load of 2.5 mg/kg was administered to each rat. The dried aqueous extracts were dissolved in distilled water. After 30 minutes of the glucose load each animal in the test groups (II-VI) was given one milliliter each of the aqueous extract at a dose of 5, 10, 20, 40 and 60 mg/kg body weight by the method described by Perez Gutierrez.2012. Distilled water (1 ml/kg) was administered for group I after the 30 minutes of the glucose load served as the control group. The rats were grouped and labeled as below.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Distilled water (1 ml/kg) treated control group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>Rats treated with 5, 10, 20, 40 and 60 mg/kg of AE of C. speciosus (n = 30)</td>
</tr>
<tr>
<td>Group III</td>
<td>Rats treated with 5, 10, 20, 40 and 60 mg/kg of AE of P. foetida (n = 30)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Rats treated with 5, 10, 20, 40 and 60 mg/kg of AE of A. carambola (n = 30)</td>
</tr>
<tr>
<td>Group V</td>
<td>Rats treated with 5, 10, 20, 40 and 60 mg/kg of AE of O. octandra (n = 30)</td>
</tr>
<tr>
<td>Group VI</td>
<td>Rats treated with 5, 10, 20, 40 and 60 mg/kg of AE of F. racemosa. (n = 30)</td>
</tr>
</tbody>
</table>
3.2.10 Evaluation of anti-hyperglycemic activity in glucose loaded normal Wistar rats

Forty two rats belongs to seven groups (n = 6) were used in this study. The 80ME and HE extracts of *C. speciosus*, *P. foetida* and *O. octandra* were investigated for their anti-hypoglycemic activity on normoglycemic rats. The dried extracts were dissolved in distilled water. A glucose load 2.5 g/kg dose was given and the extracts were administered after 30 minutes at a dose of 20 mg/kg. Distilled water (1 ml/kg) was administered for group I after the 30 minutes of the glucose load served as the control group. The rats were grouped and labeled as below.

- **Group I** - Control (distilled water; 1 ml/kg)
- **Group II** - Rats treated 20 mg/kg of *C. speciosus* (80ME)
- **Group III** - Rats treated 20 mg/kg of *C. speciosus* (HE)
- **Group IV** - Rats treated 20 mg/kg of *P. foetida* (80ME)
- **Group V** - Rats treated 20 mg/kg of *P. foetida* (HE)
- **Group VI** - Rats treated 20 mg/kg of *O. octandra* (80ME)
- **Group VII** - Rats treated 20 mg/kg of *O. octandra* (HE)
3.2.11 *In-vivo* detail activity profile of the partitioned fractions in normal rats

i. Anti-hyperglycemic activity of partitioned fractions in normal Wistar rats

Forty two rats belongs to seven groups (n = 6) were used in this study. The dried partitioned fractions (HF, CF, EF and BF) were dissolved in distilled water. A glucose load 2.5 g/kg dose was given and the fractions were administered after 30 minutes at a dose of 20 mg/kg. Distilled water (1 ml/kg) was administered for group I after the 30 minutes of the glucose load served as the control group. The rats were grouped and labeled as below.

- **Group I** - Control (distilled water; 1 ml/kg)
- **Group II** - Rats treated 20 mg/kg of HF fraction
- **Group III** - Rats treated 20 mg/kg of CF fraction
- **Group IV** - Rats treated 20 mg/kg of EF fraction
- **Group V** - Rats treated 20 mg/kg of BF fraction
- **Group VI** - Rats treated 10 mg/kg of glipizide
- **Group VII** - Rats treated 100 mg/kg of metformin
ii. The effect of pretreatment fractions on biochemical parameters in normal and alloxan induced NIDDM Wistar rats for 42 days

In the experiment, a total of 54 rats (24 normal; 30 alloxan-diabetic rats) were used. The rats were divided into nine groups (n=6). The rats were grouped and labeled as below.

Group I - Consisted of normal rats administered with distilled water 1 ml/kg

Group II - Consisted of alloxan-treated diabetic rats administered with distilled water 1 ml/kg.

Group III-V- Consisted of normal rats administered with HF, EF and BF fraction of *C. speciosus* leaves (20 mg/kg), respectively.

Groups V-VIII- Consisted of alloxan-treated diabetic rats administered with HF, EF and BF fraction of *C. speciosus* leaves (20 mg/kg), respectively.

Group IX- Consisted of alloxan-treated diabetic rats administered with glipizide (10 mg/kg).

Single dose of solution was administered every day orally using intragastric tube/sondi needle for 42 days. After 42 days of administration, animals were decapitated, blood was collected and serum was separated
immediately. Biochemical parameters were tested as in the procedures
given under the sub sections given below.

iii. Collection of blood and determination of blood glucose levels

Blood samples were collected from the tip of the tail of the rats before
administration and after 90 minutes of the administration of
extracts/fractions/drugs. Blood glucose level was determined using a
Bionime Glucometer GM300 (Bionime Corporation, Bionime GmbH,
Switzerland).

iv. Induction of non-insulin-dependent diabetes mellitus
(NIDDM)

NIDDM was induced in overnight fasted adult male rats weighing 150–
200 g by a single intraperitoneal injection of 150 mg/kg alloxan
monohydrate (Sigma Aldrich, UK) dissolved in normal saline to each rat
according to the body weight. Hyperglycemia was confirmed by the
elevated blood glucose levels determined at 72 h. Animals with blood
glucose level more than 250 mg/dl were considered as diabetic. Rats found
with permanent NIDDM were used for the antidiabetic study. This model
has been used in earlier studies to induce type II diabetes in rats (Adeneye
et al., 2007 and Venkatesh et al., 2010). Glipizide (10 mg/kg) was used as
the standard drug.
v. **Determination of total cholesterol (TC)**

Serum cholesterol levels were determined by the mechanism in which cholesterol esterase in the reagent solution cleaves cholesterol esters in the serum to release cholesterol and free fatty acids. The free cholesterol and the enzyme liberated cholesterol are then oxidized by cholesterol oxidase to form cholestenone and hydrogen peroxide ($\text{H}_2\text{O}_2$). The peroxidase catalyses the reaction between the $\text{H}_2\text{O}_2$, 4-aminophenazone and phenol to form a pink coloured complex [4-($p$-benzo-quinonemonoimino)-phenazone]. To estimate total cholesterol TC concentration, 1 ml reagent solution was added to 10 µl sample in 10 mm X 75 mm disposable tubes, mixed and then incubated at ambient temperature (25° C) for 10 min. The absorbance was read at 500 nm within 1 hour. The cholesterol concentration was calculated according to,

\[
\text{TC concentration (mg/100 ml)} = 575 \times (\text{OD 500}) \text{ sample}
\]

The serum TC concentration was expressed as mg/dL.
vi. **Determination of serum high density lipoprotein cholesterol (HDL-C)**

To 100 µL of serum was added 250 µL of precipitating solution (0.55 mmol/L, phosphotungstic acid and 25 mmol/L, MgCl₂) in order to precipitate chylomicrons, very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C). The mixture was vortex-mixed and left to stand for 10 min at ambient temperature before centrifugation for 15 min at 1500 g. The HDL-C in the supernatant was determined for its cholesterol content as previously described for TC. The serum HDL-C concentration was calculated using the formula:

\[
\text{HDL-C concentration (mg/100 ml) = 219.2} \times \text{(OD500) sample}
\]

The serum HDL-C concentration was expressed as mg/dL.

vii. **Determination of serum triglycerides (TG)**

An aliquot (1.0 ml) of reagent solution was added to 10 µL of sample, vortex mixed and incubated at 25° C for 10 min before reading the absorbance at 500 nm. The TG in the sample was hydrolyzed by lipase to liberate free fatty acid and glycerol. Glycerol kinase in the reagent converted glycerol to glycerol-3-phosphate, which in turn was oxidized by glycerol phosphate oxidase to form dihydroxyacetone phosphate and H₂O₂. Peroxidase would then catalyze the enzymatic reaction of H₂O₂
with 4-aminophenazone and 4-chlorophenol to form the pink 4-(p-benzoquinone-mono-imino)-phenazone, which absorbs at 500 nm. The following was used to calculate TG concentration:

\[
\text{TG concentration (mg/100 ml)} = 760 \times (\text{OD}_{500}) \ \text{Sample}
\]

The serum TG concentration was expressed as mg/dL.

viii. **Determination of low density lipoprotein cholesterol (LDL-C)**

The LDL-C was determined by the Friedewald’s formula (Friedewald et al., 1972).

\[
\text{LDL-C} = \text{TC} - \text{HDL-C} - \frac{\text{TG}}{5}
\]

The LDL-C concentration was expressed as mg/dL.

ix. **Determination of Anti-Atherogenic index (AAI)**

A low level of circulating HDL-C is a surrogate marker for an atherogenic metabolic situation which is commonly known as the metabolic syndrome and several strands of evidence indicate that at a low level of circulating high density lipoprotein may be related to the development of atherosclerosis (Brunzell et al., 1983). Hence, the AAI was calculated by the following formula;
FIDLC

\[ AAI = \frac{\text{HDL C}}{\text{TC} - \text{HDL C}} \times 100 \]

x. **Determination serum insulin**

Serum insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) procedure using Mercodia rat insulin ELISA kit (Asiri Central Hospital, Colombo). Briefly, the solid phase two-site enzyme immunoassay is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants (epitopes) on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtitration well. After washing three times, unbound enzyme labeled antibody was removed. The bound conjugated insulin was detected by reacting with 3, 3', 5, 5'-tetramethylbenzidine. The reaction was stopped by adding acid to give a colorimetric end-point and optical density was measured with a microplate auto reader (Shimatzu™ corporation, Japan) at a wavelength of 450 nm.

The serum insulin was expressed as \( \mu g/L \).
xi. Determination of glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin (HbA1c) was determined by ion exchange method. It has been defined operationally as the fast fraction hemoglobins HbA1 (HbA1a, A1b, A1c) which elute first during column chromatography. The non-glycosylated hemoglobin, which consists of the bulk of hemoglobin, has been designated as HbA0. A hemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the hemolysate preparation and during the binding. During this mixing, HbA0 binds to the ion exchange resin leaving HbA1c free in the supernatant. After the mixing period (five minutes), a filter separator is used to remove the resin from the supernatant. The percent glycosylated hemoglobin is determined by measuring ratio of the absorbance of the Glycosylated hemoglobin and the total hemoglobin fraction of the Control and the Test is used to calculate the percent Glycosylated hemoglobin (HbA1c) of the sample.

Hemolysate Preparation

The lysing Reagent (0.5 ml) was dispensed into tubes labeled as Control (C) and Test (T). Transferred a volume of 0.1ml of the reconstituted control (C) and well-mixed blood sample (T), into the appropriately labeled tubes. The resultant was mixed until complete lysis is evident and allowed to stand for 5 minutes before the next step.
Glycosylated hemoglobin (HbA1c) Separation

A 0.1 ml of the hemolysate was introduced into the appropriately labeled ion exchange resin tubes (Insert a resin separator into each tube so that the rubber sleeve was approximately 1 cm above the liquid level of the suspension). The tubes were mixed on vortex mixer continuously for five minutes. The resin was allowed to settle until the resin is firmly packed. The aspirate solution was poured in to each supernatant and measured each absorbance against distilled water.

Total Hemoglobin (THb) fraction

Dispensed 5.0 ml of distilled water into tubes labeled as Control (C) and Test (T). A 0.02 ml of hemolysate was added and mixed and measured each absorbance against distilled water. The percentage of THb content was calculated as follows,

\[
\text{Ratio of Control (RC)} = \frac{\text{Absorbance of control GHB}}{\text{Absorbance of control THb}}
\]

\[
\text{Ratio of Test (RT)} = \frac{\text{Absorbance of test GHB}}{\text{Absorbance of test THb}}
\]

\[
\text{THb in \%} = \frac{\text{Ratio of Test (RT)}}{\text{Ratio of Control (RC)}} \times 10 \text{(Value of Control)}
\]
xii. Evaluation of serum creatinine and determination of renal function of the pretreatment active fraction on rat model

Concentration of creatinine was determined using the method of Tietz et al. 1994. Estimating renal function, tested in rats were determined by modified using the Cockcroft-Gault equation (Katherine, 2009). Acute renal failure was induced in rats within 24 h by administrating a single dose of acetaminophen at a dose of 750 mg/kg (Palani et al., 2009). A two-compartment model was used to calculate estimating renal function.

3.2.12 Evaluation of in vitro anti-oxidant activity of active fractions

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a common organic chemical compound in the determination of in-vitro anti-oxidant activity. It is a dark-colored crystalline powder composed of stable free-radical molecules.

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH, with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPH and as consequence the absorbance’s decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most
accepted model for evaluating the free radical scavenging activity of any new drug/plant extract. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present.

The effective concentration of sample required to scavenge DPPH radical by 50% (IC50 value) will be obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations. The following assay procedure was modified from those described by Blois, 1958 and Yamasaki et al., 1994.

1. Dissolve extracts/fractions of plant materials in absolute ethanol.
2. Dilute sample by at least 5 concentrations (two-fold dilution).
3. Prepare 6 × 10^{-5} M of DPPH in absolute ethanol.
4. Transfer 500 μl of each sample solution into an eppendorf tube. Each concentration will be tested in triplicate.
5. Transfer 500 μl of DPPH solution to the mixture with sample.
6. Shake and stand at the room temperature for 30 minutes.
7. Measure the absorbance at 517 nm, using a mixture of 500 μl sample solution (test) and 500 μl ethanol as blank (control).

The DPPH free radical scavenging activity was calculated using the following formula:
% scavenging = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{\text{Absorbance of control}} \times 100

3.2.13 Statistical analysis

Results are expressed as Mean ± SEM. The data are analyzed by one way ANOVA followed by Turkey-Kramer Multiple Comparison test. Confidence Interval has been considered as 95% and p<0.05 are considered significant.
4.0 RESULTS AND DISCUSSION

4.1 Ethno-medicinal survey

The presented survey on medicinal plants used in the treatment of diabetes mellitus in Sri Lanka was conducted based on semi-structured questioner. The questioner was introduced to Ayurvedic and traditional practitioners in Sri Lanka. Where 54 practitioners were interviewed for the selection of medicinal plants after interviewing them. A total of 33 plant species were categorized, covering three major districts Kurunagala, Dambulla and Kandy in Sri Lanka. Five plants have found in relatively high frequencies in the traditional medicine for the treatment of diabetes mellitus in Sri Lanka. The present study concentrates on these plants used in human medicine. The botanical family and their frequency of use are detailed in Table 4.1.

In this study, we focused mainly on plant species reported by the local traditional practitioners in and around the study area. Present data are the general results of the ethnopharmacological survey conducted from October to December in 2009. Among all the species, Costus speciosus (74.2%), Osbeckia octandra (58.1%), Arverrhoa carambola (54.8%), Ficus racemosa (45.2%), Salacia reticulate (45.2%), Passiflora foetida (38.7%), Scoparia dulcis (22.6%) are commonly used medicinal plants by the ayurvedic and traditional practitioners for the treatment of diabetes in Sri Lanka (Figure 4.1).
The medicinal species belong to 24 different botanical families (Figure 4.2), those with the highest number of species being Cucurbitaceae and Fabaceae (12.5%), Malvaceae, Menispermaceae, Moraceae and Myrtaceae (8.3%). These families are well represented in the studied conducted by previous investigators throughout the Asian countries. Plants with more than 40% frequency values were selected. Among them Salacia reticulate L., and Scoparia dulcis L., has been omitted for further investigation as these plant have been extensively studied previously. However Costus speciosus L., Osbeckia octandra L., Passiflora foetida L., Averrhoa carambola L. and Ficus recurvosa were investigated for preliminary hypoglycemic activity.
### Table 4.1: Results of ethnopharmacological survey of medicinal plants used in the treatment of Diabetes Mellitus in Sri Lanka

<table>
<thead>
<tr>
<th>Plant name (English)</th>
<th>Local name (English)</th>
<th>Family</th>
<th>Method of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artocarpus heterophyllus (L.)</td>
<td>Kos (Jack fruit leaves)</td>
<td>Moraceae</td>
<td>DC</td>
</tr>
<tr>
<td>Averrhoa carambola (L.)</td>
<td>Kamaranka (Fruit Star Apple)</td>
<td>Oxalidaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Azadirachta indica (L.)</td>
<td>Kohomba (Neem)</td>
<td>Meliaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Cassia auriculata (L.)</td>
<td>Ranawara (Tanners Cassia)</td>
<td>Fabaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Catharanthus roseus (L.)</td>
<td>Mini mal (Vinca)</td>
<td>Apocynaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Cissampelos pareira (L.)</td>
<td>Diayamiththa (Velvet Leaf)</td>
<td>Menispermaceae</td>
<td>AE</td>
</tr>
<tr>
<td>Coccinia grandis (L.)</td>
<td>Kowakka (Ivy gourd)</td>
<td>Cucurbitaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Coscinium fenestratum (L.)</td>
<td>venivael-gaeta (False calumba or Tree Turmeric)</td>
<td>Menispermaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Costus speciosus (L.)</td>
<td>Thebu (Crape ginger leaves)</td>
<td>Costaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Cucumis callosus (L.)</td>
<td>Gon kakiri (Weed melon fruit)</td>
<td>Cucurbitaceae</td>
<td>DE</td>
</tr>
<tr>
<td>Cucumis longa (L.)</td>
<td>Kaha (Turmeric)</td>
<td>Zingiberaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Cyperus rotundus (L.)</td>
<td>Kalandru ala (Nut grass rhizome)</td>
<td>Cyperaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Eugenia malaccensis (L.)</td>
<td>Jambu (Malay apple fruit and leaves)</td>
<td>Myrtaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Ficus racemosa (L.)</td>
<td>Aththikka (Fig leaves)</td>
<td>Moraceae</td>
<td>DC</td>
</tr>
<tr>
<td>Gymnema sylvestre (L.)</td>
<td>Mas-bedda (Periplocas of the woods)</td>
<td>Asclepiadaceae</td>
<td>DC</td>
</tr>
<tr>
<td>Hibiscus tiliaceus (L.)</td>
<td>Beli kola (Cottonwood, Green Cottonwood leaves)</td>
<td>Malvaceae</td>
<td>AE</td>
</tr>
<tr>
<td>Litsea longifolia (L.)</td>
<td>Rath keliya (Endemic to Sri Lanka)</td>
<td>Lauraceae</td>
<td>DE</td>
</tr>
<tr>
<td>Mangifera indica (L.)</td>
<td>Mango (Mango seed)</td>
<td>Anacardiaceae</td>
<td>PW</td>
</tr>
<tr>
<td>Momordica charantia (L.)</td>
<td>Karawila (Bitter Gourd leaves and fruit)</td>
<td>Cucurbitaceae</td>
<td>AE</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Common Name</td>
<td>Family</td>
<td>Extract Type</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>--------</td>
<td>--------------</td>
</tr>
<tr>
<td><em>Musa balbisiana</em> (L.)</td>
<td>Alu kesel ala (Wild banana rhizome)</td>
<td>Musaceae</td>
<td>SE</td>
</tr>
<tr>
<td><em>Osbeckia octandra</em> (L.)</td>
<td>Heenbovitiya (Endemic to Sri Lanka)</td>
<td>Melastomataceae</td>
<td>FL</td>
</tr>
<tr>
<td><em>Panax fruticosum</em> (L.)</td>
<td>Koppa kola (Cut-leaved panax leaves)</td>
<td>Araliaceae</td>
<td>DC</td>
</tr>
<tr>
<td><em>Passiflora foetida</em> (L.)</td>
<td>Wal-wal Dodam (love-in-a-mist leaves)</td>
<td>Passifloraceae</td>
<td>FL and DC</td>
</tr>
<tr>
<td><em>Phyllanthus emblica</em> (L.)</td>
<td>Nelli (emblic)</td>
<td>Phyllanthaceae</td>
<td>DC</td>
</tr>
<tr>
<td><em>Pterocarpus marsupium</em> (L.)</td>
<td>Gammalu (Indian Kino seeds and leaves)</td>
<td>Fabaceae</td>
<td>BE</td>
</tr>
<tr>
<td><em>Salacia reticulata</em> (L.)</td>
<td>Kothala Himbutu (Salacia Reticulata)</td>
<td>Celastraceae</td>
<td>FL and DC</td>
</tr>
<tr>
<td><em>Scoparia dulcis</em> (L.)</td>
<td>Wal-kottamalli (Bitterbroom or licorice weed)</td>
<td>Scrophulariaceae</td>
<td>FL and DC</td>
</tr>
<tr>
<td><em>Sida veronicaefolia</em> (L.)</td>
<td>Babila mul (Country Mallow roots)</td>
<td>Malvaceae</td>
<td>DC</td>
</tr>
<tr>
<td><em>Strychnos potatorum</em> (L.)</td>
<td>Engini ata (Clearing Nut)</td>
<td>Loganiaceae (Strychnaceae)</td>
<td>AE</td>
</tr>
<tr>
<td><em>Syzygium cuminiskeels</em> (L.)</td>
<td>Madan (Black plum or Java plum)</td>
<td>Myrtaceae</td>
<td>DC</td>
</tr>
<tr>
<td><em>Trianthema portulacastrum</em> (L.)</td>
<td>Sarana (Black pig weed)</td>
<td>Aizoaceae</td>
<td>LF and AE</td>
</tr>
<tr>
<td><em>Tribulus terrestris</em> (L.)</td>
<td>Gokatu mul (caltrop roots)</td>
<td>Zygophyllaceae</td>
<td>LE and AE</td>
</tr>
<tr>
<td><em>Trigonella foenum-graecum</em> (L.)</td>
<td>Uluhaal (Fenugreek seed)</td>
<td>Fabaceae</td>
<td>LE and AE</td>
</tr>
</tbody>
</table>

DC- decoction, DE dry extract, AE- aqueous extract, PW-powder SE-semi-solid extract, FL-fresh leaves BE-bark extract
Figure 4.2: Frequency of plant families in the treatment of Diabetes Mellitus in Sri Lanka

Figure 4.3: Frequency of plant parts used in the treatment of Diabetes Mellitus in Sri Lanka
Remedies were mainly prepared in the form of powder, infusion or decoction (Table 4.1). Traditional practitioners used various plant parts (Figure 4.3) and units of measurement such as fingered length (e.g. for root, root bark, and stem), pinch (e.g. for powdered plant parts) and numbers (e.g. for leaves, seeds, fruits and flowers) were used to estimate and fix the dosage of the medicine. The methods of administration of herbal medicines in the treatment of diabetes were either as a single plant or mixtures of two or more plants, boiled to concentrate (aqueous extract). However, this study showed that plants leaves were used in significantly (p<0.05) high percentage 38.0% (Figure 4.3).

4.2 Extraction of plant material

The percentage yield of plant material extracted, using water, n-hexane and methanol, are given in Table 4.2. The yield of plant materials extracted into water ranged from 0.18% to 0.30% with the average of about 0.22%. The n-hexane soluble and methanol soluble materials were ranged from 0.12% to 0.32% and 0.27% to 0.38%. The average of n-hexane soluble and methanol yield represents 0.18% and 0.30% respectively. This showed the yield of methanol extract is more than that of aqueous and n-hexane extracts, with the exceptions in F. racemosa (0.32% yield in n-hexane).
Table 4.2: Percentage yield of water, methanol and n-hexanesoluble plant extracts

<table>
<thead>
<tr>
<th>Extraction/fraction</th>
<th>Water soluble (%)</th>
<th>n-hexane soluble (%)</th>
<th>Methanol soluble (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Costus speciosus</strong></td>
<td>0.21%</td>
<td>0.15%</td>
<td>0.38%</td>
</tr>
<tr>
<td><strong>Passiflora foetida</strong></td>
<td>0.30%</td>
<td>0.12%</td>
<td>0.31%</td>
</tr>
<tr>
<td><strong>Averrhoa carambola</strong></td>
<td>0.18%</td>
<td>0.16%</td>
<td>0.27%</td>
</tr>
<tr>
<td><strong>Osbeckea octandra</strong></td>
<td>0.22%</td>
<td>0.13%</td>
<td>0.31%</td>
</tr>
<tr>
<td><strong>Ficus racemosa</strong></td>
<td>0.18%</td>
<td>0.32%</td>
<td>0.25%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>0.22%</td>
<td>0.18%</td>
<td>0.30%</td>
</tr>
</tbody>
</table>

4.3 Dose response studies

4.3.1 Aqueous extracts

Effect on the dose of aqueous extracts on blood glucose levels (BGL) was studied in selected plants viz., *C. speciosus, P. foetida, A. carambola, O. octandra* and *F. racemosa* in rats. For this study 5.0, 10.0, 20.0, 40.0 and 60 mg/kg doses were selected and administered orally. The percentage BGL reductions were recorded when compared to the control group (distilled water). This effect was regarded as the extent of hypoglycemia.

In normal rats, *C. speciosus* aqueous extract at 20 mg/kg produced maximum hypoglycemia (59.3%) when compared to control rats (distilled water) (Table 4.3). However, of the five doses tested, 40 mg/kg and 60 mg/kg, of extract were also found to be effective, producing 55.4% and 54.0% hypoglycemic activity respectively. The maximum hypoglycemic effect of aqueous extract of *P. foetida*
and *O. otandra* exhibited (37.5% and 40.5% respectively) at the dose of 20 mg/kg when compared to control rats. The results clearly shown the activity of *C. spesiosus*, *P. foetida* and *O. otandra* extracts after 90 minutes of administration of the aqueous extract at a dose of 20 mg/kg produced significant (p>0.05) hypoglycemia more than any of the five doses of the extract used i.e., 5.0, 10.0, 20.0, 40.0 and 60 mg/kg. However the aqueous extracts of *A. carambola* and *F. racemosa* showed maximum hypoglycemia at the dose of 60 mg/kg (25.0% and 19.3% respectively).

The hypoglycemic activity of *C. spesiosus*, *P. foetida* and *O. otandra* gradually increased up to 20 mg/kg and thereafter decreased or levelled off. Whereas hypoglycemia produced by *A. carambola* and *F. racemosa* gradually increased throughout five doses studied (Figure 4.4). This result showed that the blood glucose lowering activities of *A. carambola* and *F. racemosa* was significantly lower (p<0.05) than that of *C. spesiosus*, *P. foetida* and *O. otandra* extracts. Therefore, aqueous extracts of *C. spesiosus*, *P. foetida* and *O. otandra* may produce potent hypoglycemic activity than that of *A. carambola* and *F. racemosa*. Hence, the three plants namely *C. spesiosus*, *P. foetida* and *O. otandra* were further investigated for their activity profile.

Experiments conducted to assess the dose response studies of aqueous extracts of *C. spesiosus*, *P. foetida* and *O. otandra* showed that 20 mg/kg elicits maximum hypoglycemic effect, indicating this to be the optimum dose for further investigation. Beyond 20 mg/kg, the activity was decreased or levelled off.
significantly (p<0.05) when compared to 20 mg/kg. It is possible that the aqueous extracts may stimulate a biochemical response in the target organs gradually and elicit maximum response at a dose of 20 mg/kg. Thereafter, an increase in dose does not stimulate further changes, except *C. spesiosus*, as indicated by decreasing BGL in *P. foetida* and *O. otandra*. In this study it was also observed *C. spesiosus*, *P. foetida* and *O. otandra* extracts had hypoglycemic activity above 35% whereas leaves of *A. carambola* and *F. racemosa* producing below 35%. *A. carambola* and *F. racemosa* showed non-significant (p>0.05) BGL lowering activity than that of *C. spesiosus*, *P. foetida* and *O. otandra*. Therefore the further study was focused on the activities of *C. spesiosus*, *P. foetida* and *O. otandra* in detail.
<table>
<thead>
<tr>
<th>Plant/group</th>
<th>0.0</th>
<th>5.0</th>
<th>10.0</th>
<th>20.0</th>
<th>40.0</th>
<th>60.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (Control)</td>
<td>146.4±2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Costus speciosus</em></td>
<td>75.3±6.1</td>
<td>117.6±4.4b</td>
<td>95.9±6.0b</td>
<td>59.6±4.0b</td>
<td>65.3±3.8b</td>
<td>67.4±4.4b</td>
</tr>
<tr>
<td></td>
<td>(19.7% a)</td>
<td>(34.5% a)</td>
<td>(59.3% a)</td>
<td>(55.4% a)</td>
<td>(54.0% a)</td>
<td></td>
</tr>
<tr>
<td><em>Passiflora foetida</em></td>
<td>80.4±2.2</td>
<td>118.4±2.4b</td>
<td>114.2±6.1b</td>
<td>91.5±5.6b</td>
<td>110.6±2.6b</td>
<td>115.0±1.8b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(22.0% a)</td>
<td>(37.5% a)</td>
<td>(24.5% a)</td>
<td>(21.4% a)</td>
</tr>
<tr>
<td><em>Averrhoa carambola</em></td>
<td>75.3±7.9</td>
<td>146.3±3.5</td>
<td>125.8±4.1b</td>
<td>123.1±8.7b</td>
<td>116.7±3.7b</td>
<td>109.8±2.4b</td>
</tr>
<tr>
<td></td>
<td>(0.1% a)</td>
<td></td>
<td>(14.1% a)</td>
<td>(15.9% a)</td>
<td>(20.3% a)</td>
<td>(25.0% a)</td>
</tr>
<tr>
<td><em>Osbeckia octandra</em></td>
<td>78.4±4.9</td>
<td>138.6±4.7</td>
<td>127.1±6.2b</td>
<td>101.7±2.4b</td>
<td>112.5±2.7b</td>
<td>114.3±2.8b</td>
</tr>
<tr>
<td></td>
<td>(5.3% a)</td>
<td></td>
<td>(13.2% a)</td>
<td>(40.5% a)</td>
<td>(33.2% a)</td>
<td>(21.9% a)</td>
</tr>
<tr>
<td><em>Ficus racemosa</em></td>
<td>76.3±8.6</td>
<td>136.9±4.6b</td>
<td>125.6±10.0b</td>
<td>123.0±2.2b</td>
<td>123.1±3.4b</td>
<td>118.1±2.9b</td>
</tr>
<tr>
<td></td>
<td>(6.5% a)</td>
<td></td>
<td>(14.2% a)</td>
<td>(16.0% a)</td>
<td>(15.9% a)</td>
<td>(19.3% a)</td>
</tr>
</tbody>
</table>

Number of rats per group = 6 each value is Mean ± SEM for six rats

a % reduction when compared to 20 mg/kg dose

b P < 0.05 by comparison with the control (distilled water)
4.3.2 Standard anti-hyperglycemic agents

Selection of a standard oral hypoglycemic agent for the study was conducted by investigating lowering of BGL metformin (a biguanide) and glipizide (a sulphonylurea) in rats. Experiments conducted to assess the dose activity of metformin and glipizide showed that 100 mg/kg of metformin and 10 mg/kg of glipizide elicits optimum therapeutic effect in rats under investigation. (Figure 4.4 and 4.5)

Figure 4.4: The dose response activity of the two standard drugs (glipizide and metformin) on Wistar rats

The logarithmic response showed the optimum activity in BGL lowering at a dose of 100 mg/kg for metformin and 10 mg/kg for glipizide (Figure 4.5). Therefore, the optimum dose for the two standard hypoglycemic agents was established for the further studies as 100 mg/kg for metformin and 10 mg/kg for glipizide.
Figure 4.5: Comparison of percentage reduction in blood glucose level vs log dose for methanol extracts of plants and the two standard drugs glipizide and metformin on Wistar rats.
4.4 The effect of hypoglycemic activities of aqueous, methanol and n-hexane extracts in normoglycemic rats

The three plants produced the highest hypoglycemic activity in aqueous extracts viz., C. speciosus, P. foetida and O. octandra were preliminarily investigated to assess comparative hypoglycemic activity in methanol and n-hexane soluble compounds. The hypoglycemic activity of extracted plant materials into water, 80% methanol and n-hexane, were compared with two standard anti-hyperglycemic agents, metformin (100 mg/kg) and glipizide (10 mg/kg).

The methanol extract of C. speciosus (80ME) showed highest hypoglycemic activity (61.1%) at 20 mg/kg (Table 4.4) when compared to the control group. At 20 mg/kg, 80 ME of P. foetida and O. octandra showed 48.6% and 57.9% reduction in BGLs in normal rats. The hypoglycemic activities of 80M of all three plants had significantly (p<0.05) high when compared to aqueous extracts and HE. The HE of O. octandra showed lowest hypoglycemic activity (36.3%) when compared to the control group. In comparison of aqueous, 80ME and HE, the highest hypoglycemic activities were observed in 80ME. The high extractable yield and the highest hypoglycemic activity may have a relationship between the chemical nature of the plants and the hypoglycemic activity. Therefore bioactivity guided solvent partitioning was carried out with 80ME of C. speciosus, P. foetida and O. octandra to determine the detail activity profile.
In this study both glipizide (10 mg/kg) and metformin (100 mg/kg) significantly (p<0.05) lowered the BGLs after 90 minutes of administration when compared to control rats (Table 4.4). The results of the study, showed that 80ME of all plants have produced significant (p<0.05) hypoglycemic activity than that of the two standard drugs viz., metformin at a dose 100 mg/kg (27.7%) and glipizide at a dose of 1 mg/kg (45.5%) respectively. Therefore, glipizide was selected as the referral standard anti-hyperglycemic agent in the present investigation.
4.5 Bio activity guided solvent portioning

The preliminary hypoglycemic activity of five plants selected based on the results obtained in ethno medical survey (Section 4.4), *C. speciosus*, *P. foetida* and *O. octandra* showed significantly (p<0.05) high activities than that of *A. carambola* and *F. racemosa*. Therefore detail activity profile of solvent partitioning fractions of 80ME of these three plants were studied. This was done to establish the scientific evidence of the activity profile of methanol soluble constituents in the plants. As these plants are consumed in the form of aqueous infusions or decoctions (Section 4.1) and assuming that all the water soluble compounds may have extracted in to 80ME. Partitioned fractions of 80ME of the three plants were investigated for their hypoglycemic activity at a dose of 20 mg/kg in normal and alloxan induced rats. Where, the activity of 20 mg/kg dose was produced significant p<0.05 hypoglycemia in dose vs response studies in section 4.3. The enzymatic parameters in correction of hypoglycemia of partitioned fractions of 80ME were also investigated at the same dose.

The bioactivity guided solvent partitioning of 80ME were carried out by sequential partitioning using non-polar to polar solvents, *n*-hexane, chloroform, ethyl acetate and *n*-butanol respectively. The results of the study showed in Table 4.5.
Table 4.5: Hypoglycemic activity of solvent partitioned fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* in normal and alloxan induced NIDDM rats

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Blood glucose levels mg/dl</th>
<th>C. speciosus</th>
<th>P. foetida</th>
<th>O. octandra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>142.2±2.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>99.5±3.1 b (30.0% a)</td>
<td>91.3±4.9 b</td>
<td>100.4±2.6 b (29.4% a)</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>113.6±4.6 b (21.1% a)</td>
<td>120.4±2.7 b (15.1% a)</td>
<td>135.2±1.9 b (4.9% a)</td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>88.9±2.7 b (37.5% a)</td>
<td>73.3±2.4 b (48.5% a)</td>
<td>82.2±2.5 b (42.2% a)</td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>71.1±2.6 b (45.4% a)</td>
<td>135.2±1.5 b (4.9% a)</td>
<td>96.1±5.3 b (32.4% a)</td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>133.3±3.5 b (6.3% a)</td>
<td>138.9±2.8 b (2.3% a)</td>
<td>140.2±2.3 (1.4% a)</td>
<td></td>
</tr>
</tbody>
</table>


Number of rats per group = 6 each value is Mean ± SEM for six rats

a % reduction when compared to 20 mg/kg dose

bP < 0.05 by comparison with the control (distilled water)

*Costus speciosus* showed the highest hypoglycemic activity (45.4%) in BF fraction. It also showed a non-significant (p>0.05) difference between hypoglycemic activities in HF and EF (30.0% and 37.5% respectively). The lowest activity in partitioned fractions of *Costus speciosus* showed with CF (20.1%) and Aq. remains (6.3%).
In *P. foetida* the highest significant (*p*<0.05) hypoglycemic activity (48.5%) was shown with the EF fraction. Whereas it showed 35.8% activity in HF. However the minimum activities were observed in CF (4.9%) and Aq. remains (2.3%).

In *O. octandra* the highest hypoglycemic activity had shown in EF (42.2%). It also observed similar non-significant (*p*>0.05) difference between hypoglycemic activities of HF and EF similar to that of *C. speciosus* (32.4% and 29.4% respectively). The Aq. remains showed the minimum activity (1.4%). Hence the further activity profile including phytochemical screening was carried out with butanol fraction of *C. speciosus* and ethyl acetate fractions of *P. foetida* and *O. octandra*.

### 4.6 Phytochemical screening

Observation of the bioactivity guided hypoglycemic activity (Table 4.7) of solvent partitioned fractions indicated that the different fractions with varied hypoglycemic activity. Different phytochemical groups are extracted by different solvents are given in Table 4.6 (Houghton and Raman, 1998).
Table 4.6: Types of phytochemicals extracted by different solvents (Hughton and Raman 1998)

<table>
<thead>
<tr>
<th>Polarity</th>
<th>Solvent</th>
<th>Chemical class extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>n-hexane</td>
<td>Waxes, fats, fixed oils and volatile oils</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>Alkaloids, aglycones and volatile oils</td>
</tr>
<tr>
<td>Medium</td>
<td>Dichloromethane</td>
<td>Alkaloids, aglycones and volatile oils</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>Alkaloids, aglycones and glycosides</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>Alkaloids, aglycones and glycosides</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Glycosides</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>Sugars, aminoacids and glycosides</td>
</tr>
<tr>
<td>High</td>
<td>Water</td>
<td>Sugars, aminoacids and glycosides</td>
</tr>
<tr>
<td></td>
<td>Aqueous acids</td>
<td>Sugars, aminoacids and bases</td>
</tr>
<tr>
<td></td>
<td>Aqueous alkali</td>
<td>Sugars, aminoacids and acids</td>
</tr>
</tbody>
</table>

Both CF and Aq. remains were found low in hypoglycemic activity when compared to activities produced by HF, EF and BF. However all partitioned fractionation produced lower hypoglycemic activates than that of original 80ME of respected plants under investigation. The cumulative hypoglycemic activities of partitioned fractions showed that all plants had more than hundred percent activity when compared to 80ME of *C. speciosus*, *P. foetida* and *O. octandra*.  

83
Table 4.7: Evaluation of the cumulative activities of partitioned fractions of eighty percent methanol extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Activity</th>
<th>C. speciosus</th>
<th>P. foetida</th>
<th>O. octandra</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% ME</td>
<td></td>
<td>52.7%</td>
<td>40.4%</td>
<td>36.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Activity</th>
<th>C. speciosus</th>
<th>P. foetida</th>
<th>O. octandra</th>
<th>Cumulative average activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>30.0%</td>
<td>35.8%</td>
<td>42.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>20.1%</td>
<td>15.1%</td>
<td>4.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>37.5%</td>
<td>48.5%</td>
<td>29.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>45.4%</td>
<td>4.9%</td>
<td>29.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aq-remains</td>
<td>6.3%</td>
<td>2.3%</td>
<td>1.4%</td>
<td>139.3%</td>
<td>106.6%</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared to the activities of 80% methanol extracts

The results showed in Table 4.7 indicated that all plant fractions of 80ME are more than 100% cumulative activities than the original 80ME.

Hydro alcoholic solvents are being scientifically proven by previous investigators as the best solvent to extract maximum phytochemicals, 80ME used in this study should contain both polar and non-polar phytochemicals.

As describe by Hughton and Raman in 1998, the activity profile of BF fraction of *C. speciosus* may be due to secondary metabolites such as glycosides, saponins and triterpenoids. Whereas, activities of *P. foetida* and *O. octandra* may be due to ethyl acetate soluble alkaloids, glycosides and aglycones (of steroidal saponins). This explained the presence of a group of aglycones that would solubilize in very
non-polar compounds into polar solvents as principle phytochemicals that may be responsible for the hypoglycemic activities of *C. speciosus*, *P. foetida* and *O. octandra*. Activities observed in HF fractions indicated that some of the secondary metabolites belong to waxes, fats, fixed oils and volatile oils may also responsible for the cumulative hypoglycemic activities of these plants extracts (Table 4.8).
Table 4.8: Preliminary phytochemical screening of fractions of 80% methanol extract of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* leaves

<table>
<thead>
<tr>
<th>SL</th>
<th>Plant metabolite</th>
<th><em>C. speciosus</em> (BF)</th>
<th><em>P. foetida</em> (EF)</th>
<th><em>O. octandra</em> (EF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Alkaloids</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Confirmatory TLC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Test for steroidal compounds</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Salkowski’s test</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lieberman’s test</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Test for phenolic compounds</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Ferric sulfate test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Test for free flavonoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reaction with sodium hydroxide</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>V</td>
<td>Test for saponins</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Froth test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Test for tannins</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formaldehyde test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Test for Phlobatannins</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Test for Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Test for free anthraquinones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Borntrager’s test)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Test for O-anthraquinone glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(Modified Borntrager’s test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--</td>
<td>Not detected</td>
<td>+</td>
<td>Present in low concentration</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>Present in moderate concentration</td>
<td>+++</td>
<td>Present in high concentration</td>
<td></td>
</tr>
</tbody>
</table>

The results presented in Table 4.8 are the preliminary phytochemical screening of the three plants *viz.*, *C. speciosus*, *P. foetida* and *O. octandra*. The BF of *C. speciosus* found to be contained a high concentration of flavonoids. It also contained alkaloids, steroidal compounds, phenolic compounds and saponins in moderate concentrations. Whereas *P. foetida* and *O. octandra* had high concentrations of alkaloids than *C. speciosus* and also
contained a moderate amounts of steroidal compounds. In EF of *O. octandra* found that it contained alkaloids, steroidal compounds, phenolic compounds and flavonoids in moderate concentrations.

### 4.7 Total saponin, total alkaloids and total phenol contents

The amounts of saponin in three fractions were determined. The results described in Table 4.9. The *C. speciosus* (BF) was found to be the richer in saponins (4.3%) when compared to all fractions studied. This may be due to flavonoidal aglycone rather than steroidal which was detected in preliminary phytochemical screening (Table 4.8)

#### Table 4.9: Total phenol, total alkaloid and total saponin contents of *Costus speciosus, Passiflora foetida* and *Osbechea octandra* leaves

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total saponin (%w/w)</th>
<th>Total alkaloids (%w/w)</th>
<th>Total phenolics (%w/w) As per GAE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. speciosus</em> (BF)</td>
<td>4.3%±0.3</td>
<td>4.5%±1.4</td>
<td>10.2%±0.6</td>
</tr>
<tr>
<td><em>P. foetida</em> (EF)</td>
<td>2.1%±0.2</td>
<td>5.4%±0.5</td>
<td>7.7%±1.3</td>
</tr>
<tr>
<td><em>O. octandra</em> (EF)</td>
<td>3.3%±0.3</td>
<td>1.2%±0.9</td>
<td>8.3%±1.1</td>
</tr>
</tbody>
</table>

All values represent amount of compounds in g per 100 g (%w/w) of the dry weight of the plant material taken
Mean ± SD for each triplicate

The modified Folin-Ciocalteu colorimetric method was conducted to determination the total phenolic content according the procedure described in the Experimental section 3.2.4.1. The results, presented in Table 4.9, showed that the total phenolic content varies among different fractions of *C. speciosus* (BF), *P. foetida* (EF) and *O. octandra* (EF) leaves under investigation. The BF of *C.*
C. speciosus was richer in phenols (10.2%) than the EFs of P. foetida and O. octandra.

Table 4.9 also showed the amount of total alkaloid in tested plant fractions determined by bromocresol green (BCG)-complex formation method. A yellow complex forms and easily extractable by chloroform at pH 4.7. The absorbance of the complex obeys Beer’s law over the concentration range of 10 μg/ml of chloroform. Spectrophotometric determination of total alkaloids with BCG is a simple and sensitive method and does not need very special equipment. The BCG can react with a certain class of alkaloids (alkaloids that have nitrogen inside their structure). But amine or amid alkaloids does not react with this reagent. The results, presented in Table 4.9, show that maximum to lowest total alkaloidal content varies among in order of P. foetida (EF)> C. speciosus (BF) > O. octandra (EF) i.e., 5.4%w/w, 4.5%w/w and 1.2%w/w respectively.
4.8 Evaluation of anti-diabetic activity of pretreatment partitioned fractions in rats for 42 days

Seven weeks after alloxan (ALX) induction of diabetes in male Wistar rats, the fasting blood glucose levels were measured. The hyperglycemic rats (blood glucose >250 mg/dl) were divided on day zero into three groups (n=6). The fasting BGL (initial) and BGL at the end of 42 day treatment were measured. Distilled water and glipizide (10 mg/kg) were administered orally once a day to control for 42 days, served as positive control groups. The treatment groups were administered HF, EF and BF of *C. speciosus*, *P. foetida* and *O. octandra*, orally once a day at a dose of 20 mg/kg for 42 days.

As shown in Table 4.9, the daily pretreatment at a dose of 20 mg/kg once a day for 42 days in ALX-diabetic rats, all plant fractions viz., *C. speciosus*, *P. foetida* and *O. octandra* showed a significant reduction in BGLs when compared with the diabetic control (p<0.05) in a dose dependent manner. However the effect produced by glipizide was significantly (p<0.05) higher than that of the anti-diabetic activities produced by all plants. ALX-treated diabetic rats showed significant (p<0.05) increase in the levels of blood glucose (> 250 mg/dl) when compared to normal rats. All plant fractions showed antidiabetic activity in dose dependent manner (Figure 4.6).
It was observed minimum (21.7%) anti-diabetic activity of *C. speciosus* was produced by EF and maximum activity (45.7%) with BF at a dose of 20 mg/kg when compared to the diabetic control rats. Oral administration of BF fraction of *C. speciosus* at a dose of 20 mg/kg showed highly significant (p<0.05) anti-diabetic activity in alloxan-induced NIDDM rats (Table 4.9).
Table 4.10: The effect of pretreatment fractions of n-hexane, ethyl acetate and n-butanol fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on normal and alloxan-induced NIDDM rats

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Control/standard</th>
<th>C. speciosus</th>
<th>P. foetida</th>
<th>O. octandra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>42-day treated</td>
<td>42-day treated</td>
<td>42-day treated</td>
</tr>
<tr>
<td>Normal</td>
<td>78.4±5.3</td>
<td>79.2±2.4</td>
<td>71.6±11.6</td>
<td>72.4±8.3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>76.1±3.9</td>
<td>257.1±3.5</td>
<td>251.6±4.5</td>
<td>252.4±6.7</td>
</tr>
<tr>
<td>Diabetic+HF</td>
<td>254.5±7.0</td>
<td>-</td>
<td>105.9±1.5</td>
<td>138.1±2.5</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td></td>
<td></td>
<td>(27.8%)</td>
<td>(2.7%)(^a)</td>
</tr>
<tr>
<td>Diabetic+HF</td>
<td>261.6±1.3</td>
<td>-</td>
<td>91.0±2.0</td>
<td>132.2±1.3</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>5.2</td>
<td></td>
<td>(31.2%)(^a)</td>
<td>(19.7%)(^a)</td>
</tr>
<tr>
<td>Diabetic+EF</td>
<td>267.3±1.3</td>
<td>-</td>
<td>95.6±2.1</td>
<td>132.6±5.8</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>3.8</td>
<td></td>
<td>(30.3%)(^a)</td>
<td>(15.7%)(^a)</td>
</tr>
<tr>
<td>Diabetic+EF</td>
<td>256.8±7.3</td>
<td>-</td>
<td>96.9±1.9</td>
<td>111.8±5.2</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td></td>
<td></td>
<td>(21.7%)(^a)</td>
<td>(18.4%)(^a)</td>
</tr>
<tr>
<td>Diabetic+EF</td>
<td>255.4±9.2</td>
<td>-</td>
<td>87.1±1.2</td>
<td>109.4±2.5</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td></td>
<td></td>
<td>(41.3%)(^a)</td>
<td>(36.7%)(^a)</td>
</tr>
<tr>
<td>Diabetic+EF</td>
<td>267.3±13.</td>
<td>-</td>
<td>72.9±2.4</td>
<td>103.9±6.7</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>2</td>
<td></td>
<td>(40.3%)(^a)</td>
<td>(35.9%)(^a)</td>
</tr>
<tr>
<td>Diabetic+BF</td>
<td>251.8±5.6</td>
<td>-</td>
<td>99.2±2.5</td>
<td>208.4±11.1</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td></td>
<td></td>
<td>(27.4%)(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Diabetic+BF</td>
<td>249.4±11.</td>
<td>-</td>
<td>76.6±1.6</td>
<td>201.6±9.9</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>4</td>
<td></td>
<td>(45.7%)(^a)</td>
<td>(3.3%)(^a)</td>
</tr>
<tr>
<td>Diabetic+BF</td>
<td>267.4±8.3</td>
<td>-</td>
<td>71.9±1.7</td>
<td>194.2±6.4</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td></td>
<td></td>
<td>(42.0%)(^a)</td>
<td>(3.4%)(^a)</td>
</tr>
<tr>
<td>Diabetic+glipizide</td>
<td>249.3±7.8</td>
<td>68.6±1.2</td>
<td>(73.3%)(^a)</td>
<td>-</td>
</tr>
</tbody>
</table>

Number of rats per group = 6 each value is Mean ± SEM for six rats
\(^a\) % reduction when compared to control diabetic group
\(^b\) \(P < 0.05\) by comparison with the control (distilled water)

In *O. octandra* treated rats showed highly significant \((p<0.05)\) 40.3% antidiabetic effect in 42 days when administration of EF at a dose of 20 mg/kg. The BF of *O. octandra* showed relatively lowest activity. However, EF of *P. foetida* produced 36.7% reduction of BGL at a dose of 20 mg/kg and 19.7% reduction with HF. *P.
*P. foetida* and *O. octandra* showed comparatively lower activity than that of BF. All EF indicated relatively higher anti-diabetic activities. A repeated administration of glipizide (10 mg/kg) once a day for 42 days produced 68.6% anti-diabetic activity which was a significant (p<0.05) reduction when compared to diabetic control.

As the effect of BF fraction at a dose of 40 mg/kg was less than that of 20 mg/kg dose for 42 days of all pant fractions. The dose of 20 mg/kg was selected for further the investigation of biochemical study in the correction of hyperglycemia. *C. speciosus* (BF), *P. foetida* (EF) and *O. octandra* (EF) were selected as the most active fractions for further investigation on the activity profile.

### 4.9 The evaluation of Oral Glucose Tolerance activity

Prior to Oral Glucose Tolerance activity OGTT, rats were fasted for 18 h. Distilled water (control), three selected most active fractions of *C. speciosus* (BF; 20 mg/kg), *P. foetida* (EF; 20 mg/kg) and *O. octandra* (EF; 20 mg/kg) and the reference drug, glipizide, at a dose of 10 mg/kg were orally administered to groups of six rats each. Thirty minutes later, glucose (2.5 g/kg) was orally administered to each rat with a feeding syringe. Blood samples were collected from the tail vein at 30 min (just before the administration of distilled water, fractions and glipizide in respective groups), represented 0 min. Followed by measurement of BGL at 30, 90, 180, and 270 min after glucose load.
The results presented in Table 4.10 showed the OGTT of plant fractions. *C. speciosus* BF(20 mg/kg) showed a significant (P<0.05) anti-hyperglycemic activity at 90 and 180 minutes after oral administration reducing BGLs by 44.8% and 40.1% respectively in ALX-diabetic rats. *O. octandra* EF also produced a significant (P<0.05) attenuation in BGL at 90 min (39.7 %) and 180 min (35.3 %) when compared with the diabetic control. Similarly, *P. foetida* EF (20 mg/kg) produced a significant (P<0.05) BGL reductions at 90, 180 and 270 minutes after oral administration falling BGL of 35.9 %, 20.8 % and 7.4 % respectively.
Table 4.11: Effect of anti-hyperglycemic activity by OGTT of the fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* on normal and alloxan-induced NIDDM rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose concentration mg/dl</th>
<th>0 min</th>
<th>30 min</th>
<th>90 min</th>
<th>180 min</th>
<th>270 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>76.3 ± 8.0</td>
<td>138.2 ± 8.6</td>
<td>113.5 ± 11.4</td>
<td>104.5 ± 10.2</td>
<td>78.3 ± 23.4</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>251.3 ± 8.0</td>
<td>276.8 ± 11.4</td>
<td>287.2 ± 8.6</td>
<td>266.7 ± 10.2</td>
<td>248.2 ± 23.4</td>
</tr>
<tr>
<td>Diabetic + <em>C. speciosus</em> BF(20 mg/kg)</td>
<td></td>
<td>257.3 ± 9.8</td>
<td>237.2 ± 8.6</td>
<td>186.8 ± 11.4</td>
<td>139.7 ± 10.2</td>
<td>118.2 ± 23.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(23.4 %) a,c</td>
<td>(44.8 %) a,c</td>
<td>(40.1%) a,c</td>
<td>(27.8 %) a,c</td>
</tr>
<tr>
<td>Diabetic + <em>P. foetida</em> EF(20 mg/kg)</td>
<td></td>
<td>258.7 ± 13.1</td>
<td>247.5 ± 2.3</td>
<td>108.7 ± 3.3</td>
<td>178.3 ± 5.0</td>
<td>154.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(12.2%) c</td>
<td>(35.9%) c</td>
<td>(20.8%) c</td>
<td>(7.4%) c</td>
</tr>
<tr>
<td>Diabetic + <em>O. octandra</em> EF(20 mg/kg)</td>
<td></td>
<td>251.0 ± 5.8</td>
<td>241.7 ± 6.9</td>
<td>90.9 ± 9.2</td>
<td>118.8 ± 8.2</td>
<td>121.0 ± 6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(18.7%) c</td>
<td>(39.7%) c</td>
<td>(35.3%) c</td>
<td>(29.4%) c</td>
</tr>
<tr>
<td>Diabetic + glipizide (10 mg/kg)</td>
<td></td>
<td>256.3 ± 6.2</td>
<td>191.3 ± 5.8</td>
<td>105.3 ± 7.6</td>
<td>105.3 ± 5.1</td>
<td>96.7 ± 3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(15.7%) a</td>
<td>(48.8%) a</td>
<td>(38.1%) a</td>
<td>(37.4%) a</td>
</tr>
</tbody>
</table>

Number of rats per group = 6 each value is Mean ± SEM for six rats

* % reduction when compared to alloxan-induced diabetic control group

*P < 0.05 by comparison with the normal control

*P < 0.05 by comparison with the alloxan-induced diabetic control
The glipizide caused a significant \( p<0.05 \) anti-hyperglycemic activity at 90 and 180 minutes after oral administration reducing BGLs 48.8% and 38.1% respectively in ALX-diabetic rats. It was also observed that the anti-hyperglycemic activities of *C. speciosus* (BF; 20 mg/kg), *P. foetida* (EF; 20 mg/kg) and *O. octandra* (EF; 20 mg/kg) fractions were similar to that of the anti-hyperglycemic activity produced by glipizide at a dose of 10 mg/kg throughout the study period. However, BGL lowering with glipizide treated rats were significantly \( p<0.05 \) high than that of all fractions at 270 minutes as it is known to produced hypoglycemia. However all plant fractions showed minimum anti-hyperglycemic activity after 90 minutes of the treatment. This results may be an indication that all active fractions may have been improved the hypoglycemic response to exogenous glucose load.

### 4.10 The effect of biochemical parameters in correction of hyperglycemia

The study of biochemical parameters in the correction of hypoglycemia was established studying plasma lipid profile, liver function, glycosylated hemoglobin, plasma insulin and plasma creatinine levels. Administration of some fractions under investigation brought back the levels of serum lipids to near normal. Table 4.11, Table 4.12 and Table 4.13 depict the effect produced on selected biochemical functional indices in rat serum following the repeated administration of *C. speciosus* (BF), *P. foetida* (EF) and *O. octandra* (EF) for 42 days.
Table 4.12: The effect of pretreatment of active fractions on lipid profile in normal and alloxan induced NIDDM Wistar rats for 42 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>TC</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>83.2±10.0</td>
<td>52.2±4.5</td>
<td>24.9±5.3</td>
<td>22.2±4.5</td>
</tr>
<tr>
<td>Diabetic + control</td>
<td>128.6±16.8*</td>
<td>96.6±7.3*</td>
<td>14.7±7.1*</td>
<td>55.4±9.3*</td>
</tr>
<tr>
<td>Diabetic + BF (C. speciosus 20 mg/kg)</td>
<td>98.4±4.7 b</td>
<td>59.3±9.2 b</td>
<td>27.6±6.3 b</td>
<td>33.2±11.4 b</td>
</tr>
<tr>
<td>Diabetic + EF (P. foetida 40 mg/kg)</td>
<td>115.6±14.3 ab</td>
<td>62.4±7.3 b</td>
<td>29.1±18.2</td>
<td>47.2±15.2 ab</td>
</tr>
<tr>
<td>Diabetic + EF (O. octandra 20 mg/kg)</td>
<td>101.3±6.6 ab</td>
<td>76.5±4.9 ab</td>
<td>30.7±6.2 b</td>
<td>38.4±4.9</td>
</tr>
<tr>
<td>Diabetic + glipizide (10 mg/kg)</td>
<td>97.9±26.5 ab</td>
<td>52.1±31.5 b</td>
<td>19.9±6.8 ab</td>
<td>17.8±8.8 ab</td>
</tr>
</tbody>
</table>

TC total cholesterol; TG triglycerides; HDL-C high density lipoproteins and LDL-C low density lipoproteins.
Number of rats per group = 6 each value is Mean ± SEM for six rats

*p<0.05 by comparison with the normal control

On 42nd day glipizide at a dose of 10 mg/kg reduced serum HDL-C to a value of 17.8 mg/dl which was less than that of serum HDL-C levels of normal control rats. ALT, AST and serum creatinine also produced C. speciosus (BF) administration resulted in significant (p<0.05) decrease in serum TC, TG and LDL-C by 42nd day, when compared with the ALX-diabetic control. The HDL-C concentration compared favorably when compared with both normal and ALX-diabetic rats (Table 4.12).

The Table 4.13 showed, the serum concentrations of ALT and ASL after administration of plant fractions for 42 days. C. speciosus displayed a significant (p<0.05) decrease in serum concentrations of ALT (42.1 IU/L) and ASL (41.3
IU/L) than that of both normal (78.2 IU/L) and ALX-diabetic rats (42.3 IU/L).

However the values of serum creatinine (1.3 mg/dl) were significantly (p<0.05) higher than that of normal rats (0.72 mg/dl) which was lower than that of ALX-diabetic rats (1.41 mg/dl). Which gives an indication of impaired renal functions in diabetic conditions, when treated with C. speciosus.

Table 4.13: The effect of pretreatment of fractions of Costus speciosus, Passiflora foetida and Osbeckia octandra on biochemical parameters in alloxan induced NIDDM Wistar rats for 42 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment group (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic control</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>78.2±2.1</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42.3±3.8</td>
</tr>
<tr>
<td>Serum creatinine (mg/L)</td>
<td>0.72±4.1</td>
</tr>
<tr>
<td>Serum insulin (µg/L)</td>
<td>119.2±3.2</td>
</tr>
</tbody>
</table>

Liver enzymes, ALT-Aspartate transaminase and AST- Alanine transaminase
Number of rats per group = 6 each value is Mean ± SEM for six rats

*p<0.05 by comparison with the alloxan-induced diabetic control

Administration of P. foetida (EF) showed an increase in HDL-C concentration (29.1 mg/dl) when compared to both normal and ALX-diabetic rats (Table 4.12). The serum concentrations of ALT (67.3 IU/L), after administration of P. foetida fraction, significantly (P<0.05) decreased than that of values observed with control rats (78.2 IU/L) rats. However serum ASL (58.2 IU/L) levels remain low than that of ALX-diabetic rats (42.3 IU/L). The values of serum creatinine (2.4
mg/dl) were significantly (P<0.05) higher than that of both normal rats (0.72 mg/dl) and ALX-diabetic rats (1.41 mg/dl).

When considering selected biochemical functional indices after administration of *O. octandra* showed serum concentrations of ASL as 28.1 IU/L and serum creatinine as 0.92 mg/dl.

It has observed that *C. speciosus* produced similar activities in the lipid lowering and correlation with selected biochemical functional indices with that of glipizide at 10 mg/kg dose (Table 4.11, 4.12 and 4.13) when compared to *P. foetida* and *O. octandra*.

Table 4.14: Effect of pretreatment active fractions of *Costus speciosus, Passiflora foetida* and *Osbeckia octandra* on body weight and glycosylated hemoglobin in normal alloxan-induced diabetic rats for 42 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>B.W (g/day)</th>
<th>Glycosylated Hb (%) total Hb</th>
<th>Glycosylated Hb (%) total Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Day 0</td>
<td>Final Day 42</td>
<td>Percentage change</td>
</tr>
<tr>
<td>Normal control</td>
<td>127.2±2.3</td>
<td>184.3±3.1</td>
<td>31.0%</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>138.7±6.1</td>
<td>215.3±8.3</td>
<td>35.6%</td>
</tr>
<tr>
<td>Diabetic +BF (C. speciosus 20 mg/kg)</td>
<td>126.8±3.3(^a,b)</td>
<td>187.2±6.7</td>
<td>32.3%</td>
</tr>
<tr>
<td>Diabetic + EtOAc (P. foetida 20 mg/kg)</td>
<td>131.4±0.8</td>
<td>192.4±5.3(^a,b)</td>
<td>31.6%</td>
</tr>
<tr>
<td>Diabetic + EtOAc (O. octandra 20 mg/kg)</td>
<td>122.6±5.5(^a,b)</td>
<td>210.3±8.2(^a,b)</td>
<td>41.7%</td>
</tr>
<tr>
<td>Diabetic + glipizide (10 mg/kg bw)</td>
<td>135.2±0.9</td>
<td>202.8±10.3(^a)</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

Number of rats per group = 6 each value is Mean ± SEM for six rats
\(^a\)p<0.05 by comparison with the normal control
\(^b\)p<0.05 by comparison with the alloxan-induced diabetic control
Table 4.14 and Figure 4.7 represented the effect of active fractions of C. speciosus, P. foetida and O. octandra leaves on changes in body weight and HbA1c in normal and ALX-diabetic rats. In ALX-diabetic rats, there was a significant (P<0.05) increase in body weight (55.2%) in 42 days.

Glycosylated hemoglobin had increased in ALX-diabetic rats (2.3% in normal rats to 7.4% in total Hb% in ALX-diabetic rats). However all the active fractions under investigation had reduced total Hb% than both normal and ALX-diabetic rats reflecting the overall glycemic control of all plant fractions under investigation C. Speciosus > P. foetida > O. octandra. It observed the maximum glycemic control was produced by O. octandra than C. speciosus and P. foetida.
4.11 Determination of Anti-Atherogenic index (AAI)

Figure 4.8 showed the changes of AAI in normal control, diabetic control and fractions treated diabetic rats. It is apparent from these results that the treated with BF of C. speciosus, EF of P. foetida EF and EF of O. octandra have significantly (p<0.05) altered with the AAI. The AAI value were reduced in glipizide group, in comparison with the fraction treated rats.

**Figure 4.8:** Effects of fraction treatment on Anti-Atherogenic index (AAI) in normal and alloxan-diabetic rats. AAI were plotted before (0\textsuperscript{th} day) and after daily oral treatment with vehicle (distilled water) and fractions for 42 days.
4.12 Evaluation of serum creatinine and assessment of dose dependent renal function

The maximum changes in plasma creatinine concentrations were found to occur between 6 and 20 hours post induction of ARF. Estimated GFR values obtained from the experiment were shown in Table 4.1, where the fitting factor refers to the constant in equation given in material and method section 3.2.9 when data is fitted in a rat-specific manner in Figure 4.9, as opposed to across the tested rats.

![Graph](image)

Figure 4.9: Relation of GFR with the weight (kg)/serum Creatinine (mmol/L) in rats. Number of rats per group = 6 each value is Mean ± SEM for six ra

This deviation in fitting factor gave an indication of the expected error in the GFR estimation when compared to the creatinine-clearance estimated GFR and estimation of dose dependent renal function (Katherine TM et al., 2009). Based on the calibration curve of GFR vs weight (kg)/Cr (mmol/L) the GFR for each plant fraction was estimated and presented in the Table 4.15.
Table 4.15: Effect of serum creatinine and Estimation of GFR on administration of active fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* in normal and alloxan induced NIDDM Wistar rats in the correlation of renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>Estimated GFR Normal</th>
<th>ARF induced</th>
<th>Percentage decrease in GFR</th>
<th>Fitting factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.9</td>
<td>1.6</td>
<td>44.8</td>
<td>235</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.3</td>
<td>0.7</td>
<td>46.2</td>
<td>248</td>
</tr>
<tr>
<td>Diabetic <em>C. speciosus</em></td>
<td>2.3</td>
<td>2.1</td>
<td>8.7</td>
<td>253</td>
</tr>
<tr>
<td>(20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>C. speciosus</em></td>
<td>1.9</td>
<td>1.8</td>
<td>5.3</td>
<td>264</td>
</tr>
<tr>
<td>(40 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>C. speciosus</em></td>
<td>1.3</td>
<td>1.1</td>
<td>15.4</td>
<td>271</td>
</tr>
<tr>
<td>(80 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>P. foetida</em></td>
<td>1.5</td>
<td>1.9</td>
<td>-26.7</td>
<td>266</td>
</tr>
<tr>
<td>(20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>P. foetida</em></td>
<td>1.1</td>
<td>1.5</td>
<td>-36.4</td>
<td>264</td>
</tr>
<tr>
<td>(40 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>P. foetida</em></td>
<td>0.8</td>
<td>0.9</td>
<td>-12.5</td>
<td>267</td>
</tr>
<tr>
<td>(80 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>O. octandra</em></td>
<td>2.4</td>
<td>2.3</td>
<td>4.2</td>
<td>271</td>
</tr>
<tr>
<td>(20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>O. octandra</em></td>
<td>1.6</td>
<td>2.1</td>
<td>-31.3</td>
<td>281</td>
</tr>
<tr>
<td>(40 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic <em>O. octandra</em></td>
<td>1.8</td>
<td>1.3</td>
<td>27.8</td>
<td>251</td>
</tr>
<tr>
<td>(80 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic glipizide</td>
<td>2.4</td>
<td>2.3</td>
<td>4.2</td>
<td>243</td>
</tr>
<tr>
<td>(10 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage decrease in GFR was calculated based on the reduction of GFR when compared to the initial GFR values for each group.

It has been observed that *C. speciosus*, *P. foetida* and *O. octandra* significant decrease (p<0.05) in renal function above 40 mg/kg bw dose. However all three plant fractions at 20 mg/kg bw have improved renal function when compared to diabetic rats, even though biochemical parameters showed *P. foetida* had impaired effect on liver enzymes an increase serum creatinine levels.
Figure 4.10: The effect of dose of active fractions vs GFR correlation of active fractions in normal and ARF induced rats.

Figure 4.10 showed the relationship between the dose and GFR correlation of active fractions treated rats at a dose of 20, 40 and 80 mg/kg. The ARF induced rats showed a significant (p<0.05) reduction in GFR correlations in dose dependent manner. Therefore it can be proven the maximum renal safety of these
active fractions, may be produced at a dose of 20 mg/kg for the long term treatment.

4.13 Evaluation of in-vitro DPPH anti-oxidant activity

The anti-oxidant effect of methanolic extracts of *C. speciosus* (BF), *P. foetida* (EF) and *O. octandra* (EF) showed in Table 4.16. The IC$_{50}$ values were obtained as, 6.9 to 11.2 ± 0.09 µg (*C. speciosus*), 2.0 to 85.7 ± 0.05 (*P. foetida*) and 6.6 to 13.2±0.04 (*O. octandra*) respectively when compared to IC$_{50}$ values obtained from ascorbic acid (IC$_{50}$= 8.2 to 14.4±0.11 µg).

Table 4.16: The comparison of DPPH Antioxidant activity of 80% Methanol extract of plants with their active fractions when compared to ascorbic acid

<table>
<thead>
<tr>
<th>Fraction</th>
<th>DPPH Antioxidant activity</th>
<th>r$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. speciosus</em> (80% ME)</td>
<td>3.6 to 6.4±0.04$^a$</td>
<td>0.9729</td>
</tr>
<tr>
<td><em>C. speciosus</em> (BUF)</td>
<td>6.9 to 11.2±0.09$^a$</td>
<td>0.9809</td>
</tr>
<tr>
<td><em>P. foetida</em> (80% ME))</td>
<td>6.8 to 9.7±0.33$^a$</td>
<td>0.9469</td>
</tr>
<tr>
<td><em>P. foetida</em> (EF)</td>
<td>2.0 to 85.7±0.05$^a$</td>
<td>0.4779</td>
</tr>
<tr>
<td><em>O. octandra</em> (80% ME)</td>
<td>2.6 to 4.4±0.06$^a$</td>
<td>0.9743</td>
</tr>
<tr>
<td><em>O. octandra</em> (EF)</td>
<td>6.6 to 13.2±0.04$^a$</td>
<td>0.9594</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>8.2 to 14.4±0.11$^a$</td>
<td>0.9276</td>
</tr>
</tbody>
</table>

$^a$P < 0.05 by comparison with the ascorbic acid control

The results indicated that the plant fractions of *C. speciosus* (BF) and *O. octandra* EF showed significantly (p<0.05) high antioxidant activity than that of *P. foetida* (EF). However antioxidant activities of all plant fractions produced lesser IC$_{50}$ values than that of ascorbic acid standard (Figure 4.11).
Figure 4.11: Comparison of DPPH dose dependent anti-oxidant effect of 80% Methanol extract of plants with their active fractions when compared to Ascorbic acid
4.14 Effect of food and water intakes in rats for 42 days.

The effects the active fractions on water and food intake habit of alloxan-induced hyperglycemic rats are depicted in Table 4.17 and Table 4.18 respectively.

Table 4.17: Effect of pretreatment fractions of *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* and glipizide on water intake of alloxan-induced hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water Intake Habit (ml/rat/day)</th>
<th>% Change Between Day 0 and day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Control</td>
<td>26.6±0.7</td>
<td>41.5±0.9</td>
</tr>
<tr>
<td><em>C. speciosus</em> BF (20 mg/kg)</td>
<td>28.6±0.7</td>
<td>15.3±0.6*</td>
</tr>
<tr>
<td><em>P. foetida</em> EF (20 mg/kg)</td>
<td>27.8±0.4</td>
<td>21.7±0.5*</td>
</tr>
<tr>
<td><em>O. octandra</em> (20 mg/kg)</td>
<td>29.1±0.6</td>
<td>13.8±0.4*</td>
</tr>
<tr>
<td>Glipizide (10mg/kg)</td>
<td>27.5±0.7</td>
<td>13.6±0.8*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM.; (n = 6); One way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p*<0.05 vs. Control group

*C. speciosus* BF tested at a dose of (20 mg/kg, considerably reduced food and water intake of hyperglycemic rats in comparison to diabetic control group. At the end of 42nd day, the fractions treated groups showed significant (*p*<0.05) decrease in water and food intake in comparison to diabetic control group.
Table 4.18: Effect of pretreatment fractions *Costus speciosus*, *Passiflora foetida* and *Osbeckia octandra* and glipizide on food intake of alloxan-induced hyperglycemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food Intake Habit (g/rat/day)</th>
<th>% Change Between Day 0 and day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Control</td>
<td>76.8±0.6</td>
<td>30.3</td>
</tr>
<tr>
<td><em>C. speciosus</em> BF (20 mg/kg)</td>
<td>78.8±0.7</td>
<td>-49.0</td>
</tr>
<tr>
<td><em>P. foetida</em> EF (20 mg/kg)</td>
<td>77.6±0.7</td>
<td>-17.9</td>
</tr>
<tr>
<td><em>O. octandra</em> (20 mg/kg)</td>
<td>78.6±0.6</td>
<td>-38.1</td>
</tr>
<tr>
<td>Glipizide (10mg/kg)</td>
<td>79.3±0.9</td>
<td>-49.1</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM.; (n = 6); One way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p*<0.05 vs. Control group

However the lowest values with *P. foetida* 21.9% in case of water intake and 17.9% in case of food intake were observed relative to day 0 (Before administration of fractions).
Diabetes mellitus is defined as a carbohydrate metabolic disorder characterized mainly by hyperglycemia. It may be induced by impaired insulin secretion, insulin resistance or both entities in varying proportions. It has become a major metabolic syndrome for individuals all over the world including Sri Lanka. The alarming find is that, when diagnosed type 2 diabetes, a high percentage of people already have chronic complications and/or morbid associations. Besides oral anti-hyperglycemic agents and insulin therapy, phytotherapy is an alternative source that provides a wide range of natural resources with hypoglycemic activity as per the knowledge and the practice of ayurvedic and traditional practitioners in Sri Lanka. A wide range of plant sources are recommended by them for the people with diabetes. Medicinal plants in different oral formulations in raw or as decoctions are recommended, but the mechanisms of hypoglycemic activity of them are still remains to be proven, scientifically.

Among the most popular medicinal plant species in the treatment of DM in Sri Lanka were, *Artocarpus heterophyllus* (L.), *Averrhoa carambola* (L.), *Azadirachta indica* (L.), *Coccinia grandis* (L.), *Costus speciosus* (L.), *Eugenia malaccensis* (L.), *Ficus racemosa* (L.), *Gymnema sylvestre* (L.), *Momordica charantia* (L.), *Panax fruticosum* (L.), *Passiflora foetida* (L.), *Pterocarpus marsupium* (L.), *Scoparia dulcis* (L.), *Syzygium cuminiskeels* (L.), *Trianthema portulacastrum* (L.) and *Osbeckia octandra* (L.). These medicinal plants from Sri Lankan flora, are mostly from plant families, Cucurbitaceae, Fabaceae, Malvaceae, Menispermaceae, Moraceae and Myrtaceae. Following the data obtained from the ethnomedical survey the selected plants for the scientific investigation were, namely *C. speciosus, P. foetida* and *O. octandra*. The selection
criteria of these plants were based on frequency of use by Ayurvedic and Traditional practitioners in the treatment of DM in Sri Lanka. They were studied in detail for the scientific validation on the correction of hypoglycemia on in-vivo rat models, in-vitro antioxidant activities and biochemical parameters.

The preliminary phytochemical analysis of active crude fractions, revealed that the active principles could be alkaloids, flavonoids, polyphenolic acids and/or saponins with therapeutic potential and giving hypoglycemic properties. The active fractions of 80% methanol extract of *C. speciosus*, *P. foetida* and *O. octandra* leaves were scientifically investigated for hypoglycemic and antidiabetic activity. The study showed that the preliminary hypoglycemic activity produced by extracts of *P. foetida* was significantly (p<0.05) lower than *C. speciosus* and *O. octandra*, when compared to control rats.

It observed that the blood glucose levels of active fraction treated rats were returned to the values close to the reference drug, glipizide, after the glucose challenge at 90 minutes after the treatment (Oral Glucose Tolerance Test). The evaluation of anti-hyperglycemic activity of *C. speciosus*, *P. foetida* and *O. octandra* fractions on blood glucose levels in normal and diabetic rats showed, the fractions may have the ability to improve response to exogenous glucose load. The experimental hyperglycemia was induced by intraperitoneal administration of ALX in normal rats. This effect was not reported so far from the studied plants fractions previously.

The toxic potential of *C. speciosus*, *P. foetida* and *O. octandra* were studied in-vivo sub-acute toxicity experiments in normal rats. It showed that all the fractions were reduced liver enzymes AST and ALT than diabetic rats. This may be an indication of liver enzyme
inhibition. However all fractions significantly (p< 0.05) increase serum creatinine levels and *C. spesiosus* BF fraction increased the serum insulin levels when compared to diabetic rats upon repeated administration for 42 days.

A significant decrease in total cholesterol was observed in the oral administration of *C. speciosus* and *O. octandra* for 42 days. In *P. foetida* only a slight decrease in lipid profile was observed. Therefore *C. speciosus* and *O. octandra*, could be effective to prevent or retard the development of diabetes complications due to defects in carbohydrate metabolism which leads to atherosclerosis. In this respect *C. speciosus* (BF) and *O. octandra* (EF), may be useful as antidiabetic medicinal plant with significant (p<0.05) improvement in overall lipid profile. The results on ALX-induced diabetic rats confirmed a possible potential of insulinogenic activities with *C. speciosus* (BF) and *O. octandra* (EF) as they have increased the serum insulin levels in 42 days treated rats with fractions.

The present study has revealed the antioxidant activity, with the increased DPPH scavenging activity after the treatment with BF of *C. speciosus* and EF of *O. octandra*. The ALX-induced diabetic rats were subjected to oxidative stress and that oral administration of fractions of plant fractions reduced the oxidative stress indicated by the improved biochemical parameters in the correction of hyperglycemia. Therefore, both *C. speciosus* and *O. octandra* active fraction may be useful in the prevention and the management of diabetes, atherosclerosis and the complications caused by Diabetes Mellitus.
The effects of oral administration of partitioned fractions of *C. speciosus*, *P. oetida* and *O. octandra* in in ALX-induced diabetic rats were studied. The results showed improvement on existing diabetic condition with lowering of serum total cholesterol, with an ability to stimulate antioxidant enzymatic activity including reduction of lipid peroxidation effects. However, further characterizing of BF, EF and HF fractions were not carried out due to the lack time, funding and resources. This study may provide a foundation to characterize BF, EF and HF of *C. speciosus*, *P. foetida* and *O. octandra* plant fractions. It may be worthwhile towards the identification of new chemical entities in the treatment of DM.


List of publications
