LIST OF TABLES

Table 1.1 : Current drugs for treatment of diabetes ......................... 22
Table 1.2 : Major constituents (compounds) isolated and reported from C. zeylanicum stem bark extract ................................................. 30
Table 2.1 : List of reference compounds used in the study.................. 40
Table 2.2 : Primer sequence used for cloning of various genes ............ 48
Table 2.3 : HuSH 29 mer shRNA sequences in construct against PGC1α.. 49
Table 2.4 : Optimization of transfection conditions for PPARγ Co- transfection assays ......................................................... 52
Table 2.5 : Optimization of transfection conditions for PPARα Co- transfection assays ......................................................... 53
Table 2.6 : Real-time qPCR primer sequence used for gene expression studies in HIT-T15 cells ..................................................... 58
Table 3.1 : Sub-fractionation of CZE-3 (50:50 aqueous ethanol extract).... 69
Table 3.2 : Phytochemical analysis and authentication of CZE-2 and CZE- 3 by HPLC-PDA ...................................................................... 70
Table 3.3 : Transactivation of Rosiglitazone maleate (PPARγ agonist) in HEK293/T cell based luciferase reporter assay ................. 78
Table 3.4 : Transactivation of WY-14643 (PPARα agonist) in HEK293/T cell based luciferase reporter assay ............................... 78
Table 3.5 : In vitro PPARγ transactivation potential of various extracts in transiently transfected HEK293/T cells .............................. 80
Table 3.6 : Assessment of CZE-3 mediated potentiation of PPARγ transactivation in HEK293/T cells co-transfected with PPARγ and PGC1α .............................................. 82
Table 3.7 : Effect of CZE-3 on adipogenesis in mouse 3T3-L1 cells........ 86
Table 4.1 : Effect of CZE-3 and reference compound on insulin and Pdx-1 expression in HIT-T15 cells in an acute study .................. 107
Table 5.1 : DPP-IV inhibitors approved for clinical use ................. 118
Table 5.2 : Effect of CZE-3 on DPP-IV inhibition ......................... 122
Table 5.3 : Effect of LAF-237 and MK-0431 on DPP-IV inhibition .... 123
Table 5.4 : PTP1B inhibition by CZE-3 and isolated fractions............ 128

LIST OF FIGURES

Figure 1.1 : Progression of diabetes........................................ 12
Figure 1.2 : Prevalence (%) of people with diabetes by age and sex..... 13
Figure 1.3 : Pathophysiological changes in body in diabetes........... 14
Figure 1.4 : Deaths attributable due to diabetes in age group (20-79) by IDF report 2011.......................................................... 17
Figure 1.5 : Insulin resistance and downregulation of GLUT4 in diabetes................................................................. 18
Figure 1.6 : Role of incretin hormone in glucose homeostasis........... 20
Figure 3.1 : Structure of PPAR .................................................. 62
Figure 3.2 : Gene activation by PPARs......................................... 63
Figure 3.3 : Structure of PPARγ receptor dimer bound to target DNA... 64
Figure 3.4 : Thin layer chromatography of CZE-3 (15% acetone: 85% petroleum ether)......................................................... 69
Figure 3.5 : Thin layer chromatography of CZE-2 and CZE-1........... 69
Figure 3.6 : HPLC chromatogram of compounds ............................ 71
Figure 3.7 : Analysis of 3xPPRE-pTZ57R/T clone 30 by restriction enzyme and confirmation of presence of insert .......................... 72
Figure 3.8 : Analysis of 3xPPRE-pGL3 clone 1 in 1.8% agarose gel for confirmation by mobility as compared to empty vector........... 73
Figure 3.9 : Restriction digestion analysis of 3xPPRE-pGL3 clones in 1.8% Agarose gel for confirmation of insert release .................... 73
Figure 3.10 : Cloning of PPARγ1 gene ........................................ 75
Figure 3.11 : Cloning and analysis of PPARα gene ......................... 76
Figure 3.12 : Dose-response of reference PPARγ or α agonist in HEK293/T cell based luciferase reporter assay ............................... 77
Figure 3.13 : In vitro PPARγ transactivation potential of various C. Zeylanicum extracts in HEK293/T cells ................................ 79
Figure 3.14 : Dose-response of CZE-3 in an in vitro PPARγ reporter assay in HEK293/T cells ...................................................... 80
Figure 3.15 : Dose-dependent potentiation of PPARγ transactivation by rosiglitazone and CZE-3 extract in cells co-transfected with full length PPARγ, PGC1α and 3x PPRE reporter vector ...... 81
Figure 3.16 : Effect of CZE-3 on PPARγ transactivation assay in HEK 293/T cells co-expressing SRC1 and PPARγ ............................... 83
Figure 3.17 : Reduction of compound mediated PPARγ transactivation potentiation in cells silenced with PGC1α shRNA .................. 84
Figure 3.18 : Effect of CZE extract on PPARα transactivation in HEK293/T cells ................................................................. 85
Figure 3.19 : Effect of CZE-3 and Rosiglitazone on adipogenesis in mouse 3T3-L1 cells. ............................................................... 86
Figure 3.20 : Effect of CZE-3 and Glyburide on glucose excursion in an OGT1 study in male C57BL/6J mice ................................. 88
Figure 3.21 : Effect of CZE-3 and Sitagliptin on glucose excursion in an OGT1 in male db/db mice............................................. 89
Figure 4.1 : hGPR40 reported DNA and amino acid sequence .............. 95
Figure 4.2 : 2D structure of typical 7-TM receptor ................................ 97
Figure 4.3 : Regulation of β-cell function by incretins and FFARs ........ 98
Figure 4.4 : Glucolipotoxicity induced β-cell dysfunction in diabetes .... 101
Figure 4.5 : Screening of extracts for insulin secretion potential in a glucose-dependent or independent manner in HIT-T15 cells..... 104
Figure 4.6 : Glucose-stimulated insulin secretion by reference compound GW9508 OEA and CZE extract in HIT-T15 cells .......... 104
Figure 4.7 : Dose-response of glucose-stimulated insulin secretion by GW9508 and CZE-3 in HIT-T15 cells ........................................ 104

Figure 4.8 : Effect of CZE-3 on insulin and PDX-1 expression in HIT-T15 cells in an acute study .............................................. 106

Figure 4.9 : Effect of CZE-3 on insulin mRNA expression in HIT-T15 cells treated for 90 min in presence of 15 mmol/L glucose... 108

Figure 4.10 : Effect of CZE-3 on GPR40 and GLUT2 mRNA expression in HIT-T15 cells treated for 1 h........................................... 109

Figure 4.11 : Dose-response of GW9508 in hGPR40-CHO cells in FLIPR assay ................................................................. 110

Figure 4.12 : Effect of CZE-3 on calcium-flux in hGPR40-CHO cells and wild-type CHO cells in FLIPR assay .......................... 111

Figure 4.13 : Effect of CZE-2 on calcium-flux in hGPR40-CHO cells ................................................................. 111

Figure 4.14 : Effect of CZE-3 on calcium-flux in hGPR40-CHO cells ................................................................. 112

Figure 5.1 : Physiological actions of GLP-1 and GIP and their modulation by oral DPP-IV inhibitors........................................ 116

Figure 5.2 : PCR amplification and cloning of DPP-IV in pFAST-HTa ................................................................. 119

Figure 5.3 : PCR confirmation of DPP-IV gene with bacmid DNA ................................................................. 120

Figure 5.4 : SDS-PAGE analysis of affinity purified hDPP-IV protein from SF-9 cells ................................................................. 121

Figure 5.5 : Validation of DPP-IV enzyme activity in purified protein ................................................................. 121

Figure 5.6 : DPP-IV inhibition studies by CZE-3 ................................................................. 122

Figure 5.7 : DPP-IV inhibition potential and IC_{50} determination of reference and CZE-3 ................................................................. 123

Figure 5.8 : PTP1B inhibition potential of reference and CZE-3 ................................................................. 125

Figure 5.9 : Hypothesis and probable mechanism of actions for antidiabetic effects of C.zeylanicum ................................................................. 126