STUDY ON ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF PHLOROGLUCINOL FROM BROWN MACROALGAE Padina australis Hauck

AMARCHAND CHORDIA MURUGAN

Doctor of Philosophy
(BIOTECHNOLOGY)

UNIVERSITI MALAYSIA PAHANG
SUPERVISOR’S DECLARATION

We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy (Biotechnology).

_______________________________
(Supervisor’s Signature)
Full Name : DR. NATANAMURUGARAJ GOVINDAN
Position : SENIOR LECTURER
Date : 

_______________________________
(Co-supervisor’s Signature)
Full Name : PROF. DATO’ DR. MASHITAH BINTI MOHD YUSOFF
Position : PROFESSOR
Date : 

STUDENT’S DECLARATION

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

_______________________________
(Student’s Signature)
Full Name : AMARCHAND CHORDIA MURUGAN
ID Number : PKT12001
Date : 10 AUGUST 2018
STUDY ON ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF PHLOROGLUCINOL FROM BROWN MACROALGAE Padina australis Hauck

AMARCHAND CHORDIA MURUGAN

Thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Biotechnology

Faculty of Industrial Sciences & Technology
UNIVERSITI MALAYSIA PAHANG

AUGUST 2018
ACKNOWLEDGEMENTS

This PhD thesis would not have been possible without the inspiration and support of a number of wonderful individuals, my thanks and appreciation to all of them for being part of this journey and making this thesis possible. I owe my deepest gratitude to my supervisors Dr. Natanamurugaraj Govindan and Assoc. Prof. Dr. Rezaul Karim. Without their enthusiasm, encouragement, support and continuous optimism this thesis would hardly have been completed. I express my warmest gratitude to my co-supervisor Prof. Dato’ Dr. Mashitah Binti Mohd Yusoff for her constant encouragement and support during my study. Besides my advisors, I would like to thank the Assoc. Prof. Dr. Gaanty Pragas Maniam, for his encouragement, insightful comments, and valuable inputs during the whole study. His guidance into the world of pharmacology has been a valuable input for this thesis. I also want to express my gratitude to Prof. Dr. K. Masilamani, Head, Pharmacology Department, Jaya College of Pharmacy, Chennai, Tamil Nadu, India. He has made available his support in a number of ways, especially towards the completion of this thesis.

I am forever thankful to my colleagues at the Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang for their friendship and support, and for creating a cordial working environment. I thankfully acknowledge the contributions of Science Officers of FIST laboratory & Central laboratory, UMP for taking care of all the technical, administrative matters and making it possible to carry out this work in a conducive environment. I am particularly thankful to Institute of Postgraduate Studies and Faculty of Industrial Sciences & Technology for awarding me Doctoral Scheme Scholarship.

It is a pleasure to thank my friends at the International Students Hostel, UMP for the wonderful times we shared, specially the weekend hangouts. In addition, I would like to thank all my friends in Universiti Malaysia Pahang who gave me the necessary distractions from my research and made my stay in Malaysia memorable.

Finally, my deep and sincere gratitude to my family for their continuous and unparalleled love, help and support. I am grateful to my brother Ajaypraveen Chordia Murugan and my sisters Sophia Murugan and Ashwini Murugan for always being there for me as a friend. I am forever indebted to my parents Mr. Murugan Vellaiappan and Mrs. Paranjothi Murugan for giving me the opportunities and experiences that have made me who I am. They selflessly encouraged me to explore new directions in life and seek my own destiny. This journey would not have been possible if not for them, and I dedicate this milestone to them.
Dedicated to my parents

Mr. Murugan Vellaiappan & Mrs. Paranjothi Murugan
ABSTRAK

Pencirian antidiabetik dan potensi antioksidan ekstrak polifenol dari Padina australis, rumpai laut perang telah dikaji. Ekstrak metanol (80:20 MeOH:Air suling, v/v) dari P. australis dibahagikan kepada lima bahagian dengan pelarut berbeza dan fungsi menghalang enzim α-glucosidase dan enzim α-amilase, pengekstrakan aktiviti radikal terhadap 1,1-diphenyl-2-picrylhydrazyl (DPPH), kuasa antioksida pengurangan ferik (FRAP), aktiviti kelat ion ferus (FIC) dan sifat pemutihan beta karotena telah dinilai. Pemeriksaan pencegahan antiradikal dan antidiabetik adalah signifikan secara statistik kerana ekstrak yang berbeza dari P. australis menghalang enzim secara in vitro dan in vivo. Di antara lima pecahan yang tersebut, pecahan n-butanol menunjukkan aktiviti menghambat antiradikal dan antidiabetik yang lebih tinggi berbanding dengan lain. Ekstrak metanol tertumpu kepada analisis TLC dan HPLC untuk mengenal pasti molekul bioaktif yang terdapat di dalam campuran ekstrak. Analisis LC-MS mengenai pecahan kelima n-butanol menunjukkan kehadiran tiga sebatian Phlorotannin iaitu Phloroglucinol, Eckol, dan Phlorofucofuroeckol A. Teknik kromatografi digunakan untuk mengasingkan Phloroglucinol untuk kajian selanjutnya. Konstituen aktif telah diasingkan dan dibersihkan daripada pecahan aktif dan dikaji untuk kesan antidiabetik dalam tikus diabetic Streptozotocin. Antara pecahan yang diuji, pecahan n-butanol mempunyai aktiviti penghambatan α-glucosidase dan α-amilase yang signifikan dengan nilai IC50 2.06 ± 0.14 dan 2.90 ± 0.08 mg/mL berbanding dengan Acarbose. Ketoksikan akut sebatian bulen yang dipencilkan pada dos 20 dan 40 mg/kg dalam tikus Swiss Albino mempunyai aktiviti antidiabetik yang ketara dengan peningkatan glukosa, lipid dan parameter antioksidan. Kajian toksikologi juga menunjukkan bahawa sebatian bulen Phloroglucinol tidak mempunyai kesan toksik dalam organ dalaman; berdasarkan parameter biokimia dan haematologi. Penemuan ini menunjukkan bahawa penggunaan diet Padina australis dan sebatian terpencil boleh digunakan sebagai ubat untuk rawatan kencing manis. Hasil kajian ini boleh membantu untuk membangunkan agen antihiperglisemik oral baru dalam pengurusan penyakit kencing manis (diabetes mellitus).
ABSTRACT

Diabetes is a metabolic disorder characterized by high levels of blood glucose. It is caused by the pancreatic insufficiency or by insulin resistance. Marine macroalgae extracts have been established to have strong antidiabetic and antioxidant properties. In the present study, the antidiabetic and antioxidant potential of polyphenolic extract from a brown seaweed *Padina australis* was evaluated. Methanolic (80:20 % of MeOH: Deionized Water v/v) extract of *P. australis* was partitioned with five different solvents. The carboxylic enzyme inhibiting functions, radical scavenging activity assay against 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), ferrous ion chelating (FIC) activity, beta-carotene bleaching properties are evaluated. The antiradical and antidiabetic inhibition assays were statistically significant as the different extracts of *P. australis* inhibited the enzymes in vitro and in vivo. Among the five different fractions, n-butanol fraction showed significantly higher antiradical and antidiabetic inhibitory activity. The methanolic extract was subjected to TLC and HPLC analysis to identify the bioactive molecules present in the mixture of extract. Further LC-MS analysis of n-butanol 5th fraction revealed the presence of three Phlorotannin compounds namely Phloroglucinol, Eckol, and Phlorofucofuroeckol A. Sephadex LH-20, column chromatography technique, was employed to isolate Phloroglucinol compound for further study. Phloroglucinol isolated and purified from the active fraction was evaluated for antidiabetic effect in Streptozotocin-induced diabetic rats. Among the tested portions, n-butanol fraction had significant α-glucosidase and α-amylase inhibitory activity with IC₅₀ value 2.06 ± 0.14 and 2.90 ± 0.08 mg/mL respectively, as compared to Acarbose. Acute toxicity of the isolated pure compound at 20 and 40 mg/kg doses in Swiss Albino mice was found to have potent antidiabetic activity by ameliorating glucose, lipids, and antioxidant parameters. The toxicology studies also showed that the pure compound Phloroglucinol did not have any toxic effects in the internal organs; biochemical and hematological parameters. These findings suggest that the dietary use of *Padina australis* and the isolated compound Phloroglucinol can be used as medicine for the treatment of diabetes. This work could help to develop new oral antihyperglycemic agent in the management of diabetes mellitus.
## TABLE OF CONTENT

DECLARATION

TITLE PAGE

ACKNOWLEDGEMENTS  

ABSTRAK  

ABSTRACT  

TABLE OF CONTENT  

LIST OF TABLES  

LIST OF FIGURES  

LIST OF SYMBOLS  

LIST OF ABBREVIATIONS  

CHAPTER 1 INTRODUCTION  

1.1 Introduction  

1.2 Background Statement  

1.3 Problem statements  

1.4 Research Objectives  

1.5 Scope of research  

1.6 Thesis layout  

CHAPTER 2 LITERATURE REVIEW  

2.1 Introduction  

2.2 Diabetes Mellitus  

2.2.1 Mechanism of Glucose Homeostasis in Humans
2.2.2 Health benefits of Polyphenols
2.2.3 Role of polyphenols on metabolism of carbohydrates
2.3 Diabetes-Induced Oxidative stress and use of Antioxidants
2.3.1 Oxidative stress and Reactive Oxygen Species
2.3.2 Damages Caused by Reactive Oxygen Species in Human Body
2.3.3 Oxidative stress induced diabetes complications
2.3.4 Antioxidants defense mechanism against oxidative stress
2.3.5 Epidemiological Evidence and Experimental Models
2.4 Prevalence of Diabetes Mellitus in Malaysia
2.4.1 Drug Treatment Available for Diabetes in Malaysia
2.5 Macroalgae-Derived Phlorotannins
2.5.1 Macroalgae Carbolytic Enzyme Inhibitory Activity
2.6 In Vivo Animal Model Study for Antidiabetic Effects
2.6.1 Pharmacological Induction of Diabetes Mellitus
2.6.2 Chemical Induction of Diabetes Mellitus in Animal Models
2.6.3 Streptozotocin-Induced model of Diabetes Mellitus
2.7 Summary

CHAPTER 3 METHODOLOGY

3.1 Introduction
3.2 Chemicals and Glassware
3.3 Sample Collection
3.3.1 Sample Identification
3.3.2 Preparation of Sample
3.4 Extraction of Phlorotannins
3.5 Determination of Phenolic Content and Antioxidant Properties
3.5.1 Total Phenolic Content (TPC) 28
3.5.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity 29
3.5.3 Ferric-Reducing Antioxidant Power (FRAP) 29
3.5.4 Ferrous Ion Chelating (FIC) Assay 30
3.5.5 Beta Carotene Bleaching (BCB) Assay 30

3.6 Enzyme Inhibition Study 31
3.6.1 In vitro α-Glucosidase Inhibition Assay 31
3.6.2 In vitro α-Amylase Inhibition Assay 31

3.7 Isolation and Purification 32
3.7.1 Column Chromatography 32
3.7.2 Thin Layer Chromatography Analysis 32
3.7.3 High-Performance Liquid Chromatography Analysis 32

3.8 Structure Elucidation 33
3.8.1 LC-MS Profiling 33
3.8.2 Mass Spectrometry Analysis 33

3.9 Antidiabetic Experimental Animals 33
3.9.1 Acute Toxicity Study 34
3.9.2 Streptozotocin-Induced Diabetic Rats 34

3.10 Estimation Blood and Biochemical Parameters 35
3.10.1 Estimation of Glucose 35

3.11 Estimation Protein Profile 35
3.11.1 Total Proteins 35
3.11.2 Total Albumins 36
3.11.3 Estimation of Globulins 36

3.12 Plasma Lipid Profile (Cholesterol, Triglyceride, HDL, LDL, and VLDL) 36
3.12.1 Estimation of Cholesterol 36
3.12.2 Estimation of Triglycerides 36
3.12.3 HDL-Cholesterol 37
3.12.4 LDL-Cholesterol 37

3.13 Plasma Enzymes Profile (AST, ALT, ALP, AP LDH and CPK) 37
3.13.1 Assay of Aspartate Transaminase (AST) 38
3.13.2 Assay of Alanine Transaminase (ALT) 38
3.13.3 Assay of Alkaline Phosphatase (ALP) 38
3.13.4 Assay of Acid Phosphatase (AP) 38
3.13.5 Lactate Dehydrogenase (LD) 38
3.13.6 Creatinine Phosphatase (CPK) 39

3.14 Estimation of Antioxidant Profile 39
3.14.1 Assay of Superoxide Dismutase 39
3.14.2 Assay of Catalase 39
3.14.3 Assay of Glutathione Peroxidase 40
3.14.4 Estimation of Reduced Glutathione 40
3.14.5 Estimation of Lipid Peroxidation 40

3.15 Animal Toxicological Studies 41
3.15.1 Experimental Animals 41
3.15.2 Laboratory Investigations 41
3.15.3 Hematological Investigations 41
3.15.4 Biochemical Investigations 41
3.15.5 Necropsy 42
3.15.6 Histopathology 42

3.16 Statistical Analysis 42
CHAPTER 4 RESULTS AND DISCUSSION

4.1 Introduction

4.2 Sample Collection

4.3 Extraction Yield
   4.3.1 Total Phenolic Content
   4.3.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity
   4.3.3 Ferric reducing antioxidant power (FRAP)
   4.3.4 Ferrous ion chelating (FIC) assay
   4.3.5 Beta-carotene bleaching (BCB) assay
   4.3.6 Pearson’s Correlation analysis between the total phenolic content and antioxidant capacity
   4.3.7 α-Glucosidase and α-Amylase Inhibitory Activity

4.4 Purification of Crude Extract
   4.4.1 Sephadex LH-20 Column Chromatography
   4.4.2 Yield Percentage
   4.4.3 Total Phenolic content
   4.4.4 DPPH Scavenging Activity of Purified n-Butanol Fraction
   4.4.5 α-Glucosidase and α-Amylase Inhibitory Activity

4.5 Structural Characterization of Purified Extract
   4.5.1 HPLC profiling
   4.5.2 LC-MS profile
   4.5.3 TLC Separation of n-Butanol Fraction
   4.5.4 NMR characterization

4.6 Animal Model Study for Antihyperglycemic Effect of Phloroglucinol
   4.6.1 Acute toxicity
4.6.2  Effect of Phloroglucinol on Serum Glucose Level 67
4.6.3  Effect of Phloroglucinol on Serum Protein Levels 70
4.6.4  Effect of Phloroglucinol on Serum Lipid levels 71
4.6.5  Effect of Phloroglucinol on Plasma Enzyme profiles 74
4.6.6  Effect of Phloroglucinol on Antioxidant Enzyme profile 77
4.7  Animal Toxicology Studies of Phloroglucinol 80
4.7.1  Organ weight 80
4.7.2  Haematology Investigation of Phloroglucinol Toxicity 82
4.7.3  Biochemical Parameters evaluation of Phloroglucinol Toxicity 84
4.7.4  Histopathological Examination 86
4.8  Summary 90

CHAPTER 5 CONCLUSION 91
5.1  Introduction 91
5.2  Conclusion 91
5.3  Recommendations 91

REFERENCES 93

APPENDIX A LC-MS PROFILING 110

APPENDIX B ETHICAL CLEARANCE CERTIFICATE 113

APPENDIX C LIST OF PUBLICATIONS 115
# LIST OF TABLES

| Table 2.1 | Overview of bioactive phlorotannins (polyphenols) isolated from brown marine algae. |
| Table 4.1 | Effect of different solvent extraction on the yield percentage of Padina australis Hauck |
| Table 4.2 | DPPH IC50 free radical scavenging activity of P. australis crude extracts from different solvents |
| Table 4.3 | Pearson’s correlation coefficient value between Total Phenolics and antioxidant potential of different solvent crude extracts of Padina australis Hauck. |
| Table 4.4 | α-Glucosidase inhibition activity of different solvent crude fractions of Padina australis Hauck. |
| Table 4.5 | α-Amylase inhibition activity of different solvent crude fractions of Padina australis Hauck. |
| Table 4.6 | Yield percentage of n-butanol fraction subjected to Sephadex LH-20 column chromatography. |
| Table 4.7 | Total phenolic content of purified butanol fractions of P. australis. |
| Table 4.8 | DPPH Antioxidant activity of purified n-butanol fractions of P. australis. |
| Table 4.9 | Carbolytic enzyme inhibitory activity of purified butanol fractions of Padina australis Hauck. |
| Table 4.10 | Effect of Phloroglucinol on Serum glucose level in Streptozotocin induced diabetic rats. |
| Table 4.11 | Effect of Phloroglucinol on the Serum protein level in Streptozotocin induced diabetic rats. |
| Table 4.12 | Effect of Phloroglucinol on the Total Lipid profile in Streptozotocin induced diabetic rats. |
| Table 4.13 | Effect of Phloroglucinol on the Plasma Enzyme profile in Streptozotocin induced diabetic rats. |
| Table 4.14 | Effect of Phloroglucinol on the Antioxidant enzyme profile in Streptozotocin induced diabetic rats. |
| Table 4.15 | Organ weight of Streptozocin-induced diabetic and Phloroglucinol treated mice. |
| Table 4.16 | Hematological parameters of rats in sub-acute toxicity of Phloroglucinol on 60th day. |
| Table 4.17 | Biochemical parameters of rats in sub acute toxicity of Phloroglucinol on 60th day. |
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Mechanism of glucose homeostasis</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Generation of reactive species in humans under hyperglycemic condition.</td>
<td>13</td>
</tr>
<tr>
<td>3.1</td>
<td>Location map for sample collection, Pantai Penyabong, Mersing, Johor, Malaysia</td>
<td>26</td>
</tr>
<tr>
<td>3.2</td>
<td>Collection of seaweeds from Pantai Penyabong</td>
<td>27</td>
</tr>
<tr>
<td>4.1</td>
<td>Specimen picture of Padina australis Hauck collected from rocky shores of Pantai Penyabong, Johor, Malaysia</td>
<td>44</td>
</tr>
<tr>
<td>4.2</td>
<td>Total phenolic content of different polarity solvent extracts of Padina australis Hauck (mg GAE/g).</td>
<td>46</td>
</tr>
<tr>
<td>4.3</td>
<td>DPPH free radical scavenging ability of different solvent crude extracts of P. australis</td>
<td>47</td>
</tr>
<tr>
<td>4.4</td>
<td>Ferric reducing antioxidant power (FRAP) of different solvent crude extract of P. australis</td>
<td>49</td>
</tr>
<tr>
<td>4.5</td>
<td>Ferrous ion chelating ability of different solvent crude extract of P. australis</td>
<td>50</td>
</tr>
<tr>
<td>4.6</td>
<td>Antioxidant activity of different solvent extracts of P. australis as determined by β-carotene bleaching assay</td>
<td>51</td>
</tr>
<tr>
<td>4.7</td>
<td>HPLC-DAD chromatogram of crude n-butanol extract at 254nm.</td>
<td>59</td>
</tr>
<tr>
<td>4.8</td>
<td>HPLC-DAD chromatogram of n-butanol 5th fraction at 254nm.</td>
<td>59</td>
</tr>
<tr>
<td>4.9</td>
<td>2D chemical structure of Phloroglucinol molecule isolated from P. australis</td>
<td>60</td>
</tr>
<tr>
<td>4.10</td>
<td>2D Chemical structure of Eckol molecule isolated from P. australis</td>
<td>61</td>
</tr>
<tr>
<td>4.11</td>
<td>2D Chemical structure of Phlorofucofuroeckol-A molecule isolated from P. australis</td>
<td>61</td>
</tr>
<tr>
<td>4.12</td>
<td>Thin layer chromatography separation of n-butanol 5th fraction.</td>
<td>62</td>
</tr>
<tr>
<td>4.13</td>
<td>H1 NMR profile of isolated Phloroglucinol from P. australis n-butanol 5th fraction.</td>
<td>64</td>
</tr>
<tr>
<td>4.14</td>
<td>C13 NMR profile of isolated Phloroglucinol from P. australis from n-butanol 5th fraction.</td>
<td>65</td>
</tr>
<tr>
<td>4.15</td>
<td>CDEPT NMR profile of isolated Phloroglucinol from P. australis from n-butanol fraction.</td>
<td>66</td>
</tr>
<tr>
<td>4.16</td>
<td>L.S. of Liver</td>
<td>87</td>
</tr>
<tr>
<td>4.17</td>
<td>L.S. of Kidney</td>
<td>87</td>
</tr>
<tr>
<td>4.18</td>
<td>L.S. of Brain</td>
<td>88</td>
</tr>
<tr>
<td>4.19</td>
<td>L.S. of Heart</td>
<td>88</td>
</tr>
<tr>
<td>4.20</td>
<td>L.S. of Lungs</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 4.21   L.S. of Spleen
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>α</td>
<td>alpha</td>
</tr>
<tr>
<td>β</td>
<td>beta</td>
</tr>
<tr>
<td>γ</td>
<td>gamma</td>
</tr>
<tr>
<td>%</td>
<td>percentage</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Acetyl-CoA carboxylase</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholine Esterase</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced Glycation Products</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein Kinase B (PKB)</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALS</td>
<td>Alloxan Sensitive</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
</tr>
<tr>
<td>ALX</td>
<td>Alloxan</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-Activated Protein Kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AOA</td>
<td>Antioxidant Activity</td>
</tr>
<tr>
<td>AP</td>
<td>Acid Phosphatase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Transaminase</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under Cover</td>
</tr>
<tr>
<td>BAM</td>
<td>Biologically Active Metabolite</td>
</tr>
<tr>
<td>BCB</td>
<td>Beta Carotene Bleaching</td>
</tr>
<tr>
<td>BChE</td>
<td>Butylcholine Esterase</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
</tr>
<tr>
<td>BuOH</td>
<td>Butanol</td>
</tr>
<tr>
<td>BW</td>
<td>Body Weight</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CPK</td>
<td>Creatinine Phosphatase</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic Acid</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribo Nucleic Acid</td>
</tr>
<tr>
<td>DNS</td>
<td>Dinitro Salicylic Acid</td>
</tr>
<tr>
<td>DPHC</td>
<td>Diphlorethohydroxycarmalol</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>DR</td>
<td>Degradation Rate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetraacetic Acid</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic Acid</td>
</tr>
<tr>
<td>ERK 1/2</td>
<td>Extracellular Signal-Regulated Protein Kinases 1 &amp; 2</td>
</tr>
<tr>
<td>ESR</td>
<td>Electron Spin Resonance Spectroscopy</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>FIC</td>
<td>Ferrous Ion Chelating</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric- Reducing Antioxidant Power</td>
</tr>
<tr>
<td>G6Pase</td>
<td>Glucose - 6- Phosphatase</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic Acid Equivalent</td>
</tr>
<tr>
<td>GDIP</td>
<td>Glucose-Dependent Insulinotropic Polypeptide</td>
</tr>
<tr>
<td>GIP</td>
<td>Gastric Inhibitory Polypeptide</td>
</tr>
<tr>
<td>GK</td>
<td>Goto-Kakizaki</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-Like Polypeptide-1</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose Transporter</td>
</tr>
<tr>
<td>GSH-px</td>
<td>Glutathione Peroxidase</td>
</tr>
<tr>
<td>GTG</td>
<td>Gold Thioglucose</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HCT</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipid</td>
</tr>
<tr>
<td>HGK</td>
<td>Hepatic Glucokinase</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Human Umbilical Vein Endothelial Cells</td>
</tr>
<tr>
<td>IRS</td>
<td>Insulin Receptor Substrate</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Dalton</td>
</tr>
<tr>
<td>KK</td>
<td>Kuo Kondo</td>
</tr>
<tr>
<td>KK/A_y</td>
<td>yellow KK obese</td>
</tr>
<tr>
<td>L.S.</td>
<td>Longitudinal Section</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography Coupled Mass Spectrometry</td>
</tr>
<tr>
<td>LD</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipid</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix Assisted Laser Desorption/Ionization</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>MVC</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>NO</td>
<td>Nitrous Oxide</td>
</tr>
<tr>
<td>NZO</td>
<td>New Zealand Obese</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>OLETF</td>
<td>Otuska Long Evans Tokushima Fatty</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Saline</td>
</tr>
<tr>
<td>PEPCK</td>
<td>Phosphoenolpyruvate Carboxykinase</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositide 3-Kinases</td>
</tr>
<tr>
<td>PKA</td>
<td>cAMP-Dependent Protein Kinase</td>
</tr>
<tr>
<td>PLT</td>
<td>Platelets</td>
</tr>
<tr>
<td>p-NPG</td>
<td>p-nitrophenyl α-D- glucopyranoside</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome Proliferator Activated Receptor</td>
</tr>
<tr>
<td>ppm</td>
<td>Part Per Million</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PTP</td>
<td>Phosphotyrosine Phosphatase</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmuno Assay</td>
</tr>
<tr>
<td>RLAR</td>
<td>Rat Lens Aldolase Reductase</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>SGLT</td>
<td>Sodium Glucose Co-Transporters</td>
</tr>
<tr>
<td>SGOT</td>
<td>Serum Glutamic Oxaloacetic Transaminase</td>
</tr>
<tr>
<td>SGPT</td>
<td>Serum Glutamic Pyruvic Transaminase</td>
</tr>
<tr>
<td>SHR/N-cp</td>
<td>Spontaneously Hypertensive rat/NIH-corpulent</td>
</tr>
<tr>
<td>SOD</td>
<td>Super Oxide Dismutase</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>T.S.</td>
<td>Transverse Section</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric Acid Reactive Substances</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of Flight</td>
</tr>
<tr>
<td>TPC</td>
<td>Total Phenolic Content</td>
</tr>
<tr>
<td>TSOD</td>
<td>Tsumara Suzuki Obese Diabetes</td>
</tr>
<tr>
<td>UPLC</td>
<td>Ultra Performance Liquid Chromatography</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>VMH</td>
<td>Ventromedial Hypothalamus</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>ZDF</td>
<td>Zucker Diabetic Fatty</td>
</tr>
</tbody>
</table>
"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less." - Marie Curie
REFERENCES


