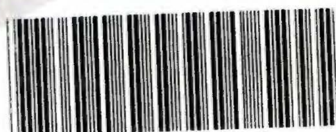
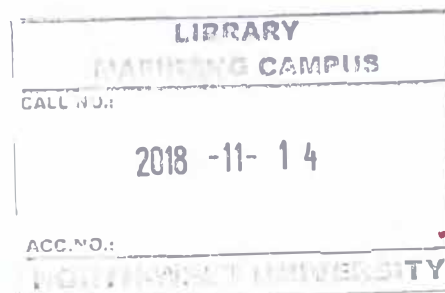


Chemical profiles, antioxidant activities and antidiabetic potential of selected South African grown cultivars of sweet potato

TAIWO BETTY AYELESO

Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Biology* at the North-West University

Supervisor: Prof E Mukwevho
Co-supervisor: Dr K Ramachela



Graduation: July 2018
Student number: 27938042

DECLARATION

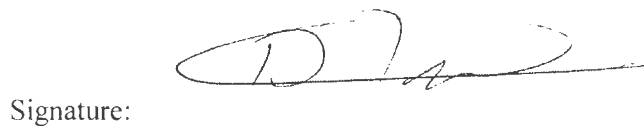
I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agricultural Sciences and the work contained herein is my original work with exception of the citations and that this work has not been submitted at any other University in part or entirety for the award of any degree.

Name: Taiwo Betty Ayeleso

Signature:

Date: 14th July, 2018

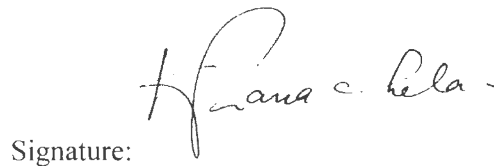
Supervisor: Prof E. Mukwevho



Signature:

Date: 13/07/2018

Co-supervisor: Dr K. Ramachela

Signature: 

Date: 16-07-2018

DEDICATION

This thesis is dedicated to almighty God who made it possible for me to successfully complete this study.

ACKNOWLEDGEMENTS

I am most grateful to almighty God for wisdom, understanding, grace, mercy and divine strength.

I sincerely appreciate my supervisor, Prof Emmanuel Mukwevho and my co-supervisor, Dr Khosi Ramachela for the opportunity, support and guidance.

Special gratitude goes to my husband, Dr. Ademola Olabode Ayeleso, words alone cannot describe how supportive, encouraging and selfless you were throughout this journey. Thank you so much! To my jewels, Abisayo Ayeleso and Abisola Ayeleso, thank you for the understanding and the sacrifices. I will also like to thank my parents, Mr Clement Adebayo Oluwagbemi and Mrs Stella Omowande Oluwagbemi for their encouragement, support and for making sure I am on the path to achieving my dreams. My gratitude also goes to my parent-in-law, Chief and Mrs P.B. Ayeleso. Thank you for being there for us always. To all my siblings, Kehinde, Aunty Dupe, Sister Nike, Idowu and especially to Motolani and my boy, Kayode, thank you for all the support.

Thanks to the families of Dr and Dr (Mrs) Aremu and Dr and Dr (Mrs) Fayemi for their love and support in one way or the other in the course of this journey.

I will also like to appreciate all my colleagues in the research group for the exchange of knowledge and time well-spent together.

Finally, I want to appreciate North-West University (NWU) for the financial support in the form of bursary throughout the duration of this study. Also, I am grateful to the National Research foundation (NRF) of South Africa for the provision of fund for this research.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES xiv

LIST OF FIGURES xvii

RESEARCH OUTPUTS xx

GENERAL ABSTRACT xxii

CHAPTER ONE 1

GENERAL INTRODUCTION 1

1.1 Background and rationale 1

1.2 The significance of the study 4

1.3 Aim and objectives of the study 4

CHAPTER TWO 6

LITERATURE REVIEW 6

2.1 OVERVIEW OF SWEET POTATO 6

2.1.1 Origin, distribution and production of sweet potato 7

NWU LIBRARY

2.1.2 Nutritional value of sweet potato....7

2.1.3 Varieties/cultivars of sweet potato....10

2.1.4 Phytochemicals in sweet potato....11

2.1.5 Medicinal potential of sweet potato....14

2.1.5.1 Antioxidant activities....15

2.1.5.2 Antidiabetic effects....16

2.1.5.3 Anticancer potential....16

2.1.5.4 Anti-ulcer potential....16

2.1.5.5 Effects on the cardiovascular system....16

2.1.5.6 Effects on the immune system....17

2.1.5.7 Antimicrobial activities....18

2.1.5.8 Anti-inflammatory potential....18

2.1.5.9 Haemotological effects....19

2.1.6 Cultivar variation in the chemical composition and bioactivities of sweetpotato....19

2.2 TYPE 2 DIABETES MELLITUS....24

2.2.1 Insulin resistance in type 2 diabetes....24

2.2.2 Glucose and fatty acid metabolism in insulin resistance....25

2.2.3 Oxidative stress and type 2 diabetes mellitus....28

2.2.4 Antioxidant defence system..31

2.2.5 The roles of carbohydrate metabolizing enzymes in type 2 diabetes..........................32

2.2.6 Phytotherapy and functional foods in the management of type 2 diabetes....................34

2.2.7 C2C12 skeletal muscle cells as an *invitro* model of insulin resistance........................35

CHAPTER THREE..37

ANALYSIS OF NUTRIENT AND CHEMICAL CONSTITUENTS OF SEVEN SOUTH AFRICAN GROWN CULTIVARS OF SWEET POTATO (*IPOMOEA BATATAS* L.)...........37

ABSTRACT..37

3.1 INTRODUCTION..38

3.2 MATERIALS AND METHODS...40

3.2.1 Preparation of tubers for analyses..40

3.2.2 Determination of protein contents...41

3.2.3 Determination of fiber contents..41

3.2.4 Determination of ash contents..42

3.2.5 Determination of fat contents...43

3.2.6 Determination of sugar contents...43

3.2.7 Determination of carbohydrates and energy values..44

3.2.8 Detection of metabolites using GC-MS...44

3.2.9 Statistical analysis...45

3.3 RESULTS...46

3.3.1 Total protein contents..46

3.3.2 Total fiber contents...48

3.3.3 Total ash contents...50

3.3.4 Total fatty acid composition...52

3.3.5 Total sugar, carbohydrates and energy values..55

3.3.6 Metabolites detected in the tubers' extracts of the cultivars..........................60

3.4 DISCUSSION..65

3.5 CONCLUSION..67

CHAPTER FOUR...68

COMPARATIVE ANALYSIS OF THE ANTIOXIDANT ACTIVITY AND PROFILES OF SELECTED SOUTH AFRICAN GROWN CULTIVARS OF SWEET POTATO.................68

ABSTRACT...68

4.1 INTRODUCTION...70

4.2 MATERIALS AND METHODS..71

4.2.1 Plant materials...71

4.2.2 Preparation of extracts...73

4.2.3 Antioxidant profiles..73

4.2.3.1 Determination of flavonol content..73

4.2.3.2 Determination of flavanal content..73

4.2.3.3 Determination of Flavonoid content..74

4.2.3.4 Determination of total polyphenol content...74

4.2.4 Antioxidant activity..75

4.2.4.1 Ferric reducing antioxidant power (FRAP) assay...75

4.2.4.2 Trolox equivalent antioxidant capacity (TEAC) assay....................................75

4.2.4.3 Nitric oxide scavenging activity assay..75

4.2.5 Quantitative liquid chromatography (LC) analysis..76

4.2.5.1 Standards and chemicals...76

4.2.5.2 Preparation of samples and standard solutions...76

4.2.5.3 Instrumentation and analysis...76

4.2.6 Statistical analysis..77

4.3 RESULTS AND DISCUSSION..78

4.3.1 Antioxidant profiles..78

4.3.2 Antioxidant activities..84

4.3.3 Correlation analysis of total polyphenol content and antioxidant activities................91

4.3.4 Qualitative analysis of specific phenolic compounds and ascorbic acid.....................95

4.4 CONCLUSION..101

CHAPTER FIVE..102

EXTRACTS OF ORANGE SWEET POTATO EXTRACTS AMELIORATE OXIDATIVE STRESS AND MODULATES DIABETES RELATED GENES IN SKELETAL MUSCLE CELLS..102

ABSTRACT..102

5.1 INTRODUCTION..104

5.2 MATERIALS AND METHODS..106

5.2.1 Cell culture..106

5.2.1.1 Storage of C2C12 cell line..106

5.2.1.2 Thawing and proliferation of C2C12 cells..106

5.2.1.3 C2C12 cells subculture and differentiation..106

5.2.2 Preparation of sweet potato samples and extracts..107

5.2.3 MTT cytotoxicity assay..108

5.2.4 Preparation of palmitate and induction of insulin resistance..109

5.2.5 Treatment of C2C12 cells with sweet potato extracts..109

5.2.6 Gene expression analysis..109

5.2.6.1 Total RNA extraction and purification..109

5.2.6.2 Agarose gel electrophoresis..110

5.2.6.3 Complimentary DNA (cDNA) synthesis...111

5.2.6.4 Quantitative transcription Polymerase Chain reaction (qRT- PCR) analysis......113

5.2.7 Oxidative stress and antioxidant activity assays...115

5.2.7.1 Protein determination assay..115

5.2.7.2 Total glutathione and glutathione peroxidase activity assays........................115

5.2.7.3 Catalase activity assay..116

5.2.7.4 Lipid peroxidation assay..118

5.2.7.5 Antioxidant activity assays...118

5.2.8 Statistical analysis..119

5.3 RESULTS..119

5.3.1 Cell proliferation and differentiation...119

5.3.2 MTT cytotoxicity assay...121

5.3.3 RNA integrity...123

5.3.4 Gene expression analysis in treated C2C12 myotubes....................................125

5.3.4.1 Effect of sweet potato extracts on expression levels of GLUT4, NRF1 and MEF2A genes......125

5.3.4.2 Effect of sweet potato extracts on expression levels of CPT1 and ACC2 genes...127

5.3.4.3 Effect of sweet potato extracts on expression level of antioxidant genes..........129

5.3.5 Effect of sweet potato extracts on oxidative stress parameters and antioxidant status.....131

5.3.5.1 Effect of sweet potato extracts on total glutathione status and lipid peroxidation.131

5.3.5.2 Effect of sweet potato extracts on antioxidant enzymes activity......133

5.3.5.3 Effect of sweet potato extracts on antioxidant capacity......135

5.4 DISCUSSION......137

5.5 CONCLUSION......141

CHAPTER SIX......142

EFFECTS OF EXTRACTS OF ORANGE FLESHED SWEET POTATO ON CARBOHYDRATE METABOLISING ENZYMES......142

ABSTRACT......142

6.1 INTRODUCTION......143

6.2 MATERIALS AND METHODS......144

6.2.1 Plant samples......144

6.2.2 Preparation of extracts......144

6.2.3 α-glucosidase, sucrase and maltase inhibition assay..144

6.2.4 α-Amylase inhibition assay..145

6.2.5 Statistical analysis..146

6.3 RESULTS AND DISCUSSION...147

6.4 CONCLUSION...152

CHAPTER SEVEN...153

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION.....................153

7.1 GENERAL DISCUSSION AND CONCLUSION..153

7.2 RECOMMENDATION..160

REFERENCES..161

ADDENDUM..206

LIST OF TABLES

Table 2.1: Nutritional values of sweet potato..9

Table 2.2: Pharmacological activity of sweet potato..21

Table 3.1: List of selected South African grown cultivars of sweet potato...........................40

Table 3.2: Fatty acid contents (%) of seven South African grown cultivars of sweet potato......54

Table 3.3: Sugar contents (%) of seven South African grown cultivars of sweet potato...........57

Table 3.4: Metabolites detected in seven South African grown cultivars of sweet potato.........61

Table 4.1: List of selected South African cultivars of sweet potato.....................................72

Table 4.2: Table 5.1: Mobile phase gradient elution program..77

Table 4.3: Total flavonol, flavanol, flavonoid and polyphenol contents of tubers of seven South African grown cultivars of sweet potato..82

Table 4.4: Total flavonol, flavanol, flavonoid and polyphenol contents of the leaves of seven South African grown cultivars of sweet potato...83

Table 4.5: Antioxidant capacity (FRAP and TEAC) of the tubers of seven South African grown cultivars of sweet potato..86

Table 4.6: Antioxidant capacity (FRAP and TEAC) of the leaves of seven South African grown cultivars of sweet potato..87

Table 4.7: Percentage inhibition (%) of Nitric oxide by tubers of seven South African grown cultivars of sweet potato..88

Table 4.8: Percentage inhibition of Nitric oxide by leaves of seven South African grown cultivars of sweet potato.....89

Table 4.9: Multiple reaction monitoring (MRM) transitions.....97

Table 4.10: Phenolic compounds and ascorbic acid composition of tubers of seven South African grown cultivars of sweet potato.....99

Table 4.11: Phenolic compounds and ascorbic acid composition of leaves of seven South African grown cultivars of sweet potato.....100

Table 5.1: Treatment groups for differentiated C2C12 cells.....112

Table 5.2: Yield of extracted RNA from the treatment groups and the reaction components for the synthesis of cDNA.....112

Table 5.3: Component mix for Quantitative reverse transcription polymerase chain (qRT-PCR) reactions.....114

Table 5.4: Primers sequences for Quantitative reverse transcription polymerase chain (qRT-PCR) reaction.....114

Table 5.5: Reaction mixture for glutathione peroxidase activity.....117

Table 5.6: Reaction mixture for catalase activity assay.....117

Table 5.7: Percentage viability of C2C12 cells treated with different concentrations of OSPT and OSPL.....122

Table 5.8: The effect orange fleshed sweet potato extracts on antioxidant capacity (FRAP AND TEAC VALUES)..136

Table 6.1: IC_{50} values for α-glucosidase, α -amylase, sucrase and maltase inhibitory potential of the extracts of orange fleshed sweet potato and acarbose..151

LIST OF FIGURES

Figure 2.1: Some common phytochemicals in sweet Potato..13

Figure 2.2: Fatty acid oxidation and glucose uptake in peripheral cells such as the skeletal muscle...27

Figure 2.3: Different sources of reactive oxygen species (ROS) production in Type 2 diabetes...30

Figure 3.1: Protein contents of seven South African grown cultivars of sweet potato.............47

Figure 3.2: Fiber contents of seven South African grown cultivars of sweet potato...............49

Figure 3.3: Ash contents of seven South African grown cultivars of sweet potato.................51

Figure 3.4: Energy values of seven South African grown cultivars of sweet potato...............58

Figure 3.5: Carbohydrates contents of seven South African grown cultivars of sweet potato.....59

Figure 4.1: Nitric oxide scavenging activity IC_{50} value (mg/ml) of the tubers extracts of seven South African grown cultivars of sweet potato...90

Figure 4.2: Nitric oxide scavenging activity IC_{50} value (mg/ml) of the leaves' extracts of seven South African grown cultivars of sweet potato...90

Figure 4.3: Correlation between total polyphenol content and FRAP values of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves..92

Figure 4.4: Correlation between total polyphenol content and TEAC values of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves....93

Figure 4.5: Correlation between total polyphenol content and IC_{50} values of Nitric oxide (NO) scavenging activity of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves....94

Figure 4.6: Overlapped chromatograms of standard polyphenols and ascorbic acid....98

Figure 4.7: Overlapped chromatograms of aqueous and aqueous methanol extracts of the tubers and leaves of seven South African grown sweet potato cultivars....98

Figure 5.1: C2C12 myocytes and myoblast....120

Figure 5.2: Agarose gel electrophoresis of RNA samples....124

Figure 5.3: The effect of orange fleshed sweet potato extracts on the expression of (a) Glucose transporter 4 (GLUT4) (b) Mycocyte enhance factor 2A (MEF2A) (c) Nuclear respiratory factor 1 (NRF1) in treated C2C12 myotubes....126

Figure 5.4: The effect of orange fleshed sweet potato extracts on the expression of Carnitine palmitoyl transferase 1 (CPT1) and Acetyl CoA carboxylase 2 (ACC2) in treated C2C12 myotubes....128

Figure 5.5: The effect of orange fleshed sweet potato extracts on the expression of antioxidant enzymes (a) Glutathione peroxidase (GPx) (b) Catalase (CAT) in treated C2C12 myotubes....130

Figure 5.6: The effect of orange fleshed sweet potato extracts on (a) total glutathione status and (b) lipid peroxidation (GSH) in treated C2C12 myotubes...132

Figure 5.7: The effect of orange fleshed sweet potato extracts on (a) glutathione peroxidase (GPx) activity and (b) catalase (CAT) activity in treated C2C12 myotubes...........................134

Figure 6.1: Percentage inhibition of extracts of orange fleshed sweet potato and acarbose on α-glucosidase..149

Figure 6.2: Percentage inhibition of extracts of orange fleshed sweet potato and acarbose on sucrase...149

Figure 6.3: Percentage inhibition of extracts of orange fleshed sweet potato and acarbose on maltase...150

Figure 6.4: Percentage inhibition of extracts of orange fleshed sweet potato and acarbose on α-amylase...150

RESEARCH OUTPUTS

a) Manuscript published

Chapter 2: The literature review on sweet potato in section 2.2 has been published as detailed below:

A review of therapeutic potentials of sweet potato: Pharmacological activities and influence of cultivar. Tropical Journal of Phamarceutical Research 15(12):2751-2761.

Authors: Taiwo Betty Ayeleso, Khosi Ramachela and Emmanuel Mukwevho

Candidate's Contributions: designed the study, managed the literature searches, and wrote the first draft of the manuscript.

b) Manuscripts for publications

Chapter 3: Analysis of nutrient and chemical constituents of South African grown cultivars of sweet potato (*Ipomoea batatas* l.).

Authors: Taiwo Betty Ayeleso, Khosi Ramachela and Emmanuel Mukwevho

Candidate's Contributions: co-designed the study, performed the laboratory works, interpreted the results and wrote the first draft of the manuscript.

Chapter 4: Comparative analysis of the antioxidant activity and profiles of selected South African cultivars of sweet potato submitted to *Journal of food composition and analysis.*

Authors: Taiwo Betty Ayeleso, Khosi Ramachela and Emmanuel Mukwevho

Candidate's Contributions: co-designed the study, performed the laboratory works, interpreted the results and wrote the first draft of the manuscript.

Chapter 5: Extracts of orange sweet potato extracts ameliorate oxidative stress and modulates diabetes related genes in skeletal muscle cells submitted to *Molecules*.

Authors: Taiwo Betty Ayeleso, Khosi Ramachela and Emmanuel Mukwevho

Candidate's Contributions: co-designed the study, performed the laboratory works, interpreted the results and wrote the first draft of the manuscript.

Chapter 6: Effects of extracts of orange fleshed sweet potato on carbohydrate metabolising enzymes submitted to *Journal of Herbal Medicine*.

Authors: Taiwo Betty Ayeleso, Khosi Ramachela and Emmanuel Mukwevho

Candidate's Contributions: co-designed the study, performed the laboratory works, interpreted the results and wrote the first draft of the manuscript.

c) Conference presentations

Ayeleso TB, Ramachela K and Mukwevho E. Extracts of orange fleshed sweet potato ameliorate oxidative stress in insulin-resistant C2C12 cells. Conference of the Physiological Society of South Africa (PSSA), University of Pretoria, Pretoria, 27th-31st August, 2017.

Ayeleso TB, Ramachela K and Mukwevho E. Aqueous-methanol extracts of orange fleshed sweet potato modulate type 2 diabetes associated genes in insulin-resistant C2C12 cells. SASBMB-FASBMB conference, North-West University, Potchestroom, 8th-11th July 2018.

GENERAL ABSTRACT

Sweet potato is one of the important food crops cultivated across the world especially in developing countries. In South Africa, it is one of the most important crops being promoted for food security and nutrition. Sweet potato has various cultivars characterized by different skin and flesh colours as well as variation in chemical composition. There is limited information on the nutritional composition of sweet potato cultivars grown and consumed in South Africa. Furthermore, plants including food crops are resources that are presently being explored in the development of alternative medicine for chronic diseases. Apart from the proximate and basic nutritional composition of sweet potato, it also contains beneficial phytochemicals which have therapeutic potential in different diseases. The objective of this research study was to determine the phytochemical constituents, nutritional composition, antioxidant activity and antidiabetic potential of sweet potato cultivars grown in South Africa. The cultivars of sweet potato used for this study were four (4) white fleshed (Blesbok, Ndou, Monate and Mvuvhelo) and three (3) orange fleshed (Bophelo, Impilo and 199062.1). The nutritional compositions were analyzed according to AOAC methods. Metabolites detection was carried using GC-MS analysis. Antioxidant profiles were determined spectrophotometrically, specific polyphenols were quantified using LC-MS and the antioxidant activity was carried out by using FRAP, TEAC and NO radical scavenging activity assays. Extracts (aqueous and aqueous-methanol) of 'Bophelo' were used for the *in vitro* oxidative stress assays and antidiabetic potential study. Differentiated C2C12 cells were made insulin resistant by treating with 0.75mM palmitate for 16 hours. Cells were divided into seven treatment groups; CONTROL (untreated cells), PT (palmitate only), PT+OSPT (palmitate and 500μg/ml tuber extracts), PT+OSPT (palmitate and 100μg/ml leaf extract), PT+M (palmitate and 1μM metformin), OSPT (500μg/ml tuber extracts only), OSPL

(100µg/ml leaf extracts only). Expression levels of GLUT4, NRF1, MEF2A, CPT1, ACC2 genes and antioxidant enzymes; CAT and GPx were determined by qRT-PCR analysis. Oxidative stress biomarkers; total glutathione status, lipid peroxidation and activities of antioxidant enzymes were measured using established methods. The inhibition activity of the extracts on carbohydrate metabolizing enzymes; α-glucosidase, α-amylase, sucrase and maltase were also assessed. Statistical analysis was carried out using Graph pad Prism 6 at $p<0.05$ accepted as the level of significance between treatments groups.

The highest protein and total fat values were both found in 'Bophelo' and the highest values of fiber, ash, total sugars, carbohydrates, and energy values were found in 'Impilo', '199062.1', 'Impilo', 'Blesbok' and 'Monate' respectively. The lowest protein, fiber and ash contents were noted in 'Blesbok', while the lowest values of total fat and total sugars were found in 'Impilo' and 'Ndou' respectively. The sweet potato line '199062.1' had the lowest total carbohydrates and energy values. Generally, 'Bophelo' consistently had higher antioxidant contents with the exception of total flavonoid and total flavanol in the tubers and leaves respectively. The results indicate that the average amounts of total flavonol and polyphenols are about 50 folds and 20 folds higher in the leaves than in the tubers extracts respectively. However, the flavanol contents of both the tubers and leaves remain comparable. Furthermore, the aqueous and aqueous methanol extracts of 'Bophelo' exhibited the strongest antioxidant activities in the tubers and leaves respectively as determined by FRAP and TEAC assays. Quantitative LC-MS analysis results indicate that hyperoside and caffeic acid were generally the most abundant compounds found in the leaves and tuber extracts respectively. Additionally, the aqueous and aqueous methanol extracts of the tubers and leaves of 'Bophelo' respectively were further explored for their antioxidative effects and antidiabetic potential in palmitate-induced insulin-resistant C2C12

cells. Treatments of the insulin resistant cells with the extracts of 'Bophelo' resulted into a significant ($P<0.05$) increase in intracellular GSH level, non-significant increase in CAT activity and significant reduction in the level of MDA. The intracellular antioxidant status as determined by the FRAP and TEAC methods were also improved significantly upon treatment with the extracts. The gene expression analysis showed that, in the palmitate treated groups, there were significant decreases in expressions of CAT and GPx genes which were significantly ($p<0.05$) increased upon treatment with the extracts. Similarly, there were also significant lower levels of expression of GLUT4, NRF1 and MEF2A in the palmitate treated group which were increased significantly upon treatments with the extracts. Furthermore, there was a 4.2 fold increase in expression of ACC2 in the palmitate treated group when compared with the control. This reduced significantly to approximately 3 and 2 folds upon treatment with the extracts of the tubers and leaves respectively. However, the levels of expression of all the genes in the groups treated with both palmitate and extracts were still significantly different from the untreated control group. Cells treated with extracts only (without palmitate) improved across all the parameters measured. The extracts showed significantly lesser inhibition and higher IC_{50} values of the activities of all the carbohydrate metabolising enzymes compared to acarbose at ($P<0.05$). However, the leaf extracts showed an appreciable inhibition of the enzymes and a significantly lower IC_{50} values at ($P<0.05$) when compared with the tuber extract. In conclusion, this study showed variation in the nutritional and phytochemical composition as well as antioxidant activity in sweet potato resulting from cultivar, part of plant (leaves and tubers) and extraction solvent differences. It also demonstrated the antidiabetic potential of orange fleshed sweet potato through the ability of the extracts to ameliorate oxidative stress, modulate diabetes related genes and mildly inhibit carbohydrate metabolizing enzymes.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND AND RATIONALE

Sweet potato [*Ipomoea batatas* (L.) Lam] is an important food crop being cultivated across the world especially in developing countries. It is a perennial crop which is tolerant to drought and can thrive even with high rainfall, low soil fertility and little maintenance (CIP, 1995). Sweet potato tubers are rich in carbohydrates, dietary fiber and have a low glycemic index (Astawana and Widowati, 2011). Different parts of the plant also contain mineral nutrients such as potassium, calcium, sodium, zinc, manganese, iron, magnesium and vitamins such as vitamins A, C, and B2 (Riboflavin) and lutein (Woolfe, 1992; Hue *et al.*, 2012).

Sweet potato is mostly cultivated for its tubers as a source of staple food in different parts of the world. The tubers can be fried, boiled or air/sun dried and ground into a fine powder to supplement other foods or simply added as a sweetener (Tewe *et al.*, 2003). The milled flour is also used to supplement wheat flour for baking purposes (Nnawuchi *et al.*, 2002). In some Asian countries such as China, Japan, and Korea, sweet potato tubers are usually baked or roasted and sold as street food, especially in the winter. It is also used in the production of pasta and also fermented to produce a probiotic juice. In some parts of Japan, the anthocyanin-rich purple varieties are made into sparkling liquor and wine (Yamakawa, 2000). In spite of the nutritional qualities and utilization potential of sweet potato, the consumption of sweet potato in South Africa is low in comparison to other crops such as the traditional potato. This might possibly be due to lack of awareness of its nutritional value. Sweet potato, is therefore, one of the important crops being promoted for food security and nutrition (Laurie *et al.*, 2017).

NWU
LIBRARY

Sweet potato has different varieties/cultivars, as with other food crops. Many studies have demonstrated that significant variation in nutritional composition usually exists among these cultivars even when grown under the same conditions (Padda and Picha, 2008; Ji *et al*., 2015). Sweet potato cultivars are usually characterized by their skin and flesh colours, but there can still be significant difference in nutritional composition between cultivars of same skin and flesh colours (Ayeleso *et al*., 2016).

Generally, plants contain different bioactive compounds which can interact with different metabolic processes and produce a physiological response that can result in the prevention or therapeutic management of various diseases (Tosetti *et al*., 2009; Angeloni *et al*., 2012; Upadhyay and Dixit, 2015). Therefore, plants including food crops can be explored in the development of alternative medicine for chronic diseases. The view that food can be beneficial beyond its traditional nutritional value is already attracting the attention of scientists, health professionals and consumers (Rani and Sharma 2003). Current interest in controlling and preventing degenerative diseases through nutraceuticals has promoted research on the bioactive components of edible plants (Burn and Kishore, 2000; Huang *et al*., 2007). In addition to the nutritional benefits of sweet potato, there are several literature evidences of its biological activities such as antioxidant (Hue *et al*., 2012; Fidrianny *et al*., 2013), antidiabetes (Pal *et al*., 2015; Ogunrinola *et al*., 2015) and anticancer (Karna *et al*., 2011; Sugata *et al*., 2015). These beneficial effects have been attributed to the various phytochemicals present in both the leaves and tubers of the food crop. Phytochemicals have been linked to many positive health effects against many ailments in humans and animal studies including type 2 diabetes (Barone *et al*., 2009; Lee *et al*., 2009; Rizvi and Mishra, 2009).

Type 2 diabetes mellitus is a metabolic disorder associated with episodes of hyperglycemia, glucose intolerance as a result of lack of insulin, impaired insulin action or both (Sicree *et al.*, 2006). Herbal remedy in the management of diabetes has been investigated in a large number of different plants such as Ginger (Asha *et. al.*, 2011), kidney bean (Luka *et al.*, 2013) and cucumber (Saidu *et al.*, 2014). Oxidative stress has been implicated in different metabolic disease process including diabetes (Le lay *et al.*, 2014). Hence, strategies to prevent and ameliorate oxidative stress remain important in the overall treatment of insulin resistance and type 2 diabetes (Henriksen *et al.*, 2011). A lot of food crops such as vegetables and fruits have been confirmed to contain antioxidants which can be beneficial to human health (Eastwood, 1999; Pandey and Rizvi, 2009). Apart from their basic nutritional values, food crops can as well be exploited for their antioxidants contents and possible medicinal values. A number of studies have reported the antidiabetic properties of different cultivars of sweet potato mostly by assessing blood glucose level, glucose tolerance, and lipid accumulation (Kusano and Abe, 2000, Ludvik *et al.*, 2002). However, its mechanisms of action have not been extensively explored. Investigating the effects of sweet potato extracts on oxidative stress parameters, carbohydrate metabolizing enzymes and genes/transcription factors involved in type 2 diabetes would give an insight into some of the mechanisms through which it exerts its antidiabetic effects. Therefore, this study investigated the chemical composition, antioxidant profiles and activity as well as the *in vitro* antidiabetic potential of selected sweet potato cultivars grown in South Africa. All the cultivars namely: Blesbok, Bophelo, Ndou, Monate, Mvuvhelo, Impilo and 199062.1 were cultivated and grown under the same conditions on the university farm of North-West University, Mafikeng campus, South Africa.

1.2 SIGNIFICANCE OF THE STUDY

Chemical profiling of the cultivars will provide information on the nutritional qualities of the cultivars for informed dietary advice and consumption. Breeders can also utilize this information to create sweet potato genotypes with improved nutritional profile. Furthermore, because bioactive compounds in plants may vary greatly among cultivars of the same plant, there is a need for the proper evaluation and selection of cultivars with the appropriate phytochemical composition and bioactivity. Screening of South African sweet potato cultivars for antioxidant and antidiabetic activity can lead to the development of high nutraceutical value product for the management of diabetes or generally as a dietary supplement for improved health. Moreover, this study will give an insight into the mechanisms of action of the blood glucose lowering properties of sweet potato extracts. This study can also provide the platform for the identification and isolation of certain bioactive constituents which may serve as a starting or model molecule for the production of semi or novel synthetic antidiabetic drugs.

1.3 AIM AND OBJECTIVES OF THE STUDY

This aim of this study was to investigate the phytochemical composition, antioxidant activities and antidiabetic effect of the leaves and tubers of selected South African grown cultivars of sweet potato. The specific objectives of the study include:

1. To analyse proximate and fatty acids composition of the tubers of the selected cultivars.
2. To estimate the antioxidant profiles and quantify specific flavonoids, phenolic acids and antioxidant vitamin in the extracts of the leaves and tubers using HPLC.
3. To determine the various metabolites found in the different cultivars using GCMS.
4. To evaluate the *in vitro* antioxidant activity of the extracts of the leaves and tubers using ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) and nitric oxide (NO) scavenging assays.

5. To determine the effect of the extracts on cell viability and oxidative stress biomarkers in insulin resistant C2C12 cells.
6. To evaluate the antidiabetic potential of the cultivar with the highest antioxidant capacity by screening the activity of the extracts on the carbohydrate metabolizing enzymes: alpha-amylase, alpha-glucosidase, sucrase and maltase.
7. To analyse the antidiabetic potential of the cultivar with the highest antioxidant capacity by screening the activity of the extracts on the expression levels of genes related to type 2 diabetes: NRF1, GLUT4, MEF2A, CPT1, and ACC2 in C2C12 cells.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF SWEET POTATO

Sweet potato, *Ipomoea batatas* (L.) Lam. is a perennial crop which belongs to the morning glory family or Convolvulaceae (Senanayake *et al.*, 2013). It is a popular staple food of the tropical and subtropical areas with a nutritional benefit evidenced by increase in its cultivation and consumption (Ferris *et al.*, 2002). Sweet potato is mostly harvested for its tubers. However, the leaves are also sometimes consumed as an alternative to other leafy vegetables. It is the sixth most important food crop in the world and contains phytochemicals, which are important for human health (Islam, 2014, Grace *et al.*, 2014). Other than their nutritional benefits such as a rich source of dietary fibre, antioxidants, vitamins, and minerals, sweet potato root tubers also contain no saturated fats or cholesterol. Sweet potato leaves contain more polyphenols than any other commercial vegetables such as spinach, cabbage, and lettuce with at least 15 anthocyanins and 6 polyphenolic compounds (Islam, 2014). Several reports have indicated that the phytochemicals in sweet potato possess multifaceted actions, including antioxidant, antimutagenic, antiinflammatory, antimicrobial and anticarcinogenesis and thus are important for several health-promoting functions in humans (Konczak-Islam *et al.*, 2003).

Different varieties of sweet potato are grown worldwide. These are generally characterized by different flesh colours and varying phytochemical compositions. Different varieties of a plant may inherently differ in their nutritional values and in the bioactivities of phytochemicals present in the plants (Flores *et al.*, 2015; Shekhar *et al.* 2015). The nutritional value and medicinal potentials of sweet potato are gaining the attention of many research groups as the quest for natural remedies from plants as well as the understanding between diet and health increases worldwide. Sweet potato plant,

alongside being primarily a food resource, may as well be exploited for its medicinal properties due to its high nutritive and therapeutic properties.

2.1.1 Origin, distribution and production of sweet potato

Sweet potato, although native to tropical regions in America, is an important and global food crop which is cultivated in more than 100 countries, with the primary or main commercial producers in China, Indonesia, Vietnam, Japan, India, Tanzania and Uganda (Schronk, 2010). Limpopo, Mpumalanga (Nelspruit), KwaZulu-Natal and Western Cape provinces are the major production areas in South Africa (DAFF, 2011). Sweet potato is mainly grown in developing countries which account for 95 % of the global output. China accounts for about 65 % of the world's sweet potato production. In most parts of Africa, the production of sweet potato is carried out on a small or subsistence level. Nonetheless, there are high productions in the East and West African regions i.e Rwanda, Uganda, Burundi, Congo, Nigeria, Ghana and Madagascar (Hijmans *et al*., 1999).

Sweet potato yields maximum and better quality roots on a well-drained, sandy or silt loam soil. It requires both warm days and nights for optimum growth and tuber development (Verma, 2014). Storage root development of sweet potato also depends on good soil aeration. Good soil aeration can be achieved by good field choice and bedding before transplantation. It is very sensitive to alkaline and saline conditions which influence growth. Soil pH between 5.6 and 6.6 is very good for the production of sweet potato (Laurie and Niederwieser, 2004).

2.1.2 Nutritional value of sweet potato

Sweet potatoes are rich in complex carbohydrates, dietary fiber and beta carotene (a precursor of vitamin A), vitamin B6, and vitamin C (Table 2.1). In addition to this, various parts of the crop have been reported to also contain mineral nutrients such as zinc, potassium, sodium, manganese, calcium, magnesium and iron (Woolfe, 1992). According to the Food and Agricultural Organisation (FAO)

(1990), sweet potato leaves and shoots are good sources of vitamins A, C and B2 (riboflavin), and lutein. Orange sweet potato varieties have higher beta carotene content than those with light coloured flesh and their increased cultivation is being encouraged in Africa where Vitamin A deficiency is a challenging health issue. On the other hand, purple-fleshed sweet potato has been reported to contain anthocyanins, which possess antioxidant activities (Teow *et al.*, 2007). Although the protein content of sweet potato is low (~2 %), as in most tropical root and tuber crops, sweet potato still contains more protein than cassava and plantain (Woolfe, 1992). The leaves have relatively high protein content (25-30% of dry matter) compared to other leafy vegetables. The leaves also have higher levels of polyphenols than any other commercial vegetable (Islam, 2014). Polyphenols have a strong role in the contribution to the prevention of degenerative diseases such as cancer and cardiovascular diseases through their antioxidant activities (Scalbert *et al.*, 2005).

Table 2.1: Nutritional value of sweet potato

Parameter	Nutritional value per 100 g	
	Root tuber	**Leaf**
Water	77.28	86.81
Energy (kcal)	86	42
Carbohydrates(g)	20.12	8.82
Protein (g)	1.57	2.49
Total fiber (g)	3	5.3
Total lipid (g)	0.05	0.51
Vitamins		
Vitamin A (IU)	14187	3778
Thiamine (mg)	0.078	0.156
Riboflavin (mg)	0.061	0.345
Niacin (mg)	0.557	1.130
Vitamin B6 (mg)	0.209	0.190
Folate (B9) (μg)	11	1
Vitamin C (mg)	2.4	11
Vitamin K (μg)	1.8	302.02
Minerals		
Calcium (mg)	30	78
Iron (mg)	0.61	0.97
Magnesium (mg)	25	70
Phosphorus (mg)	47	81
Potassium (mg)	337	508
Sodium (mg)	55	6
Lipids		
Fatty acids, total saturated (g)	0.018	0.111
Fatty acids, total monounsaturated (g)	0.001	0.020
Fatty acids, total polyunsaturated (g)	0.014	0.228

Source: United States Department of Agriculture National Nutrient Database (2015)

2.1.3 Varieties/cultivars of sweet potato

Currently, there are several cultivars of sweet potato known and cultivated around the world. These cultivars come in different colours, shapes, sizes and also vary in taste and texture. There are two major categories of sweet potato cultivars and these are (i) the staple types which are white, red/ purple skinned with white/cream flesh and are characterized by their high starch content (ii) the dessert types which are orange skinned, orange fleshed and are characterized by their high beta carotene content (DAFF, 2011). Different cultivars of sweet potato are also characterized by their colours, width, thickness and shapes of the leaves (Hue *et al.*, 2010).

There are several cultivated varieties of sweet potato in the United States but one of the leading and the most widely cultivated is the carrot coloured and orange-silver skinned cultivar known as Centennial. Beauregard and Jewel are also dominant varieties of sweet potato that is being popularly consumed in the United States (Tan *et al.*, 2007). In China, where sweet potato breeding is now aimed at improved nutrition in animal feed and high starch content, a large number of new cultivars are emerging each year. 'Xushu 18', which was bred for high resistance against sweet potato root rot disease caused by Fusarium solani, still remains the most cultivated variety in China (Carpena *et al.*, 2009). In sub-Saharan Africa, sweet potato is a major food crop now being bred for improved nutrition. An example is the increased production of orange cultivars to combat vitamin A deficiency (Tumwegamire *et al.*, 2004). Globally, several improved cultivars of sweet potato have been developed by collection of the best local farmer varieties as well as the introduction of best cultivars from other parts of the world. As in all other plants, the objective of breeding in sweet potato is mostly for improved nutritional benefit, high yield, long keeping quality and, resistance to pest and diseases (Kapinga *et al.*, 2007).

In Southern Africa, formal sweet potato breeding was initiated in 1952 (Bester and Louw, 1992) and the most commercially successful has been Blesbok, a purple-skinned high yielding cultivar with low

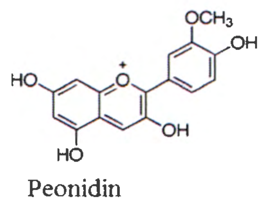
dry matter content (Bester *et al.*, 1991). Sweet potato is already an indigenized food crop being cultivated in South Africa, particularly in the northern subtropical regions of the country (Laurie *et al.*, 2017). In the last decades, sweet potato cultivars with varying characteristics have been developed by the Agricultural Research Council (ARC) breeding programmes in South Africa to address food security and nutrition. Imported lines such as Beauregard and W-119 are common cultivars which are also being grown in South Africa.

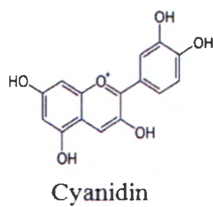
The selected cultivars used in this study are an imported line from International Potato Centre (CIP) in Lima Peru and some of the lines from ARC breeding programmes since 1989. The flesh colours of the tubers range from pale cream to dark orange (Addendum 1). Adaptability, improved root quality, high yield, sweet taste and especially beta-carotene content have been the major goals for the breeding of sweet potato cultivars in South Africa (Laurie *et al.*, 2017). Currently, there are various cultivars of sweet potato being grown and consumed in South Africa, but there is limited information on the chemical composition and nutritional qualities of these cultivars.

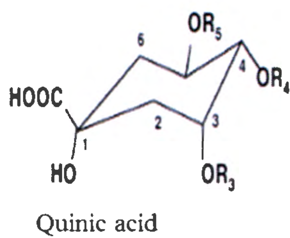
2.1.4 Phytochemicals in sweet potato

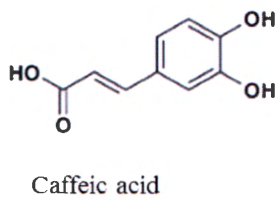
The major phytochemicals that are generally present in sweet potato are flavonoids, terpenoids, tannins, saponins, glycosides, alkaloids, steroids and phenolic acids. These constituents may vary with varieties depending on flesh and skin colours. Orange varieties are particularly rich in beta-carotene, while purple sweet potato contains higher anthocyanin content than other varieties of sweet potato (Anthoney and Omwenga, 2014). Beta carotene is a terpenoid with a strongly colored red-orange pigment abundant in plants and fruits. Anthocyanins are members of the flavonoid group of phytochemicals responsible for the red, purple and blue pigments in many fruit and vegetables (Lila, 2004). The antioxidant activities of sweet potato have mostly been attributed to their anthocyanin and beta-carotene contents.

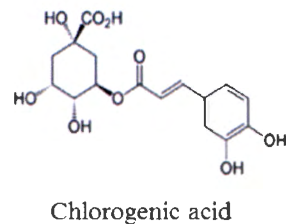
Structures of common phytochemicals in sweet potato are shown in Figure 2.1. Peonidin and cyanidin are examples of anthocyanins present in purple sweet potato. Park *et al.* (2016) conducted a metabolite profiling study on different varieties of sweet potato. They reported that peonidin and cyanidin were only detected in purple fleshed sweet potato but not in the white and orange varieties. Luteolin, a flavonoid was found in orange and purple varieties but was absent in the white ones. Phenolic acids such as chlorogenic, isochlorogenic, caffeic, cinammic, and hydroxycinammic acids are also generally present in sweet potato. Phenolic acids have been associated with colour, sensory qualities, nutritional value as well as antioxidant properties of foods (Robbins, 2003). They are more abundant in purple fleshed sweet potato than in the other colours varieties (Park *et al.*, 2016). Other important chemical compositions of sweet potato include starch, protein, vitamins, minerals and dietary fibre. The non-starch polysaccharides; hemicellulose and cellulose are also important medicinal components of sweet potato that provide protective effects against colon cancers and vascular diseases (Woolfe, 1992). Vitamin A is abundant in orange fleshed sweet potato, and hence an appropriate food source to address vitamin A deficiency (Gurmu *et al.*, 2014). High content of vitamin B6 in the tubers contribute in the reduction of the blood levels of homocysteine, an amino acid which has been implicated as a risk factor in cardiovascular diseases (Gripper, 2013).











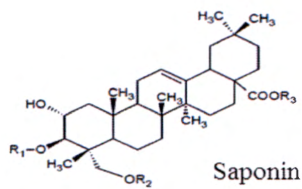


Figure 2.1: Some common phytochemicals in sweet Potato

2.1.5 Medicinal potentials of sweet potato

Many studies have reported different medicinal potentials of sweet potato. These properties have been attributed to either a single or combined effect of the phytochemicals present in the plant. In traditional medicine, sweet potato has been used to treat many diseases such as oral infections, inflammatory diseases (Anbuselvi and Balamuragan, 2014) and also in the management of diabetic conditions (Dutta, 2015). In recent times, pharmacological potential of sweet potato has been investigated and demonstrated by different *in vitro*, animal models and a few human studies (Table 2.2a-2.2c).

2.1.5.1 Antioxidant activities

Phytochemicals such as flavonoids and related phenolic compounds which are generally present in sweet potatoes have been reported to have multiple biological effects, such as antioxidant activity. Purple-fleshed variety has been reported to contain anthocyanins, which possess antioxidant activity (Teow *et al*., 2007). Antioxidants act as scavengers of free radicals mainly in the forms of reactive oxygen species inside the cell (Devasagayam *et al*., 2004). Many evidences suggest that degenerative diseases such as cancer, asthma, diabetes, senile dementia and eye disease have their origin in deleterious free radical reactions (Florence, 1995). The free radical scavenging activity of extracts from the leaves of eight (8) cultivars of sweet potato was confirmed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, the antioxidant activity of the leaves' extract correlated with the total polyphenol content (Nagai *et al*. 2011). Fidrianny *et al*. (2013) concluded from their study that n-hexane, ethylacetate and ethanol extracts of leaves from different cultivars of sweet potato showed free radicals scavenging activity. In the root tubers of sweet potato, antioxidant activity has also been demonstrated in different cultivars (Tang *et al*., 2015; Ji *et al*., 2015). A stronger antioxidant activity was reported in the peels of white and purple varieties when compared to the flesh samples (Salawu

et al., 2015), showing that the skin of sweet potato root tubers are also rich source of anti-oxidative phytochemicals.

2.1.5.2 Antidiabetic effect

There are studies that have suggested that sweet potato has the potential of lowering the blood glucose level. In some animal and human studies, sweet potato has been reported to help in maintaining blood sugar levels and lowering insulin resistance. 'Caiapo' is a dietary supplement and a crude extract of white skinned sweet potato which has been sold and consumed for a long time in Japan as a remedy for diabetes (Ludvik *et al*, 2004). 'White star' a sweet potato cultivar indigenous to Pakistan and 'Beauregard' which is indigenous to the United States lowers glucose blood level in diabetic patients (Zaikar *et al.* 2008). The leaf extract of sweet potato reduces significantly the level of blood glucose and hepatic enzymes activities in Alloxan- induced diabetic rats (Ogunrinnola *et al*., 2015). The findings are similar with the result of a recent study that reported that the aqueous extract of the leaves of sweet potato showed significant improvement in the blood glucose profile of diabetic rats (Pal *et al.* 2015). The blood glucose lowering effect of white skinned sweet potato in type 2 diabetic patients has been linked to an increase in blood levels of adiponectin; an adipocyte hormone that serves as an important modifier of insulin metabolism (Ludvik *et al*., 2008). Patients with poorly-regulated insulin metabolism and insulin insensitivity tend to have lower levels of adiponectin, and individuals with healthier insulin metabolism tend to have higher levels (Lindsay *et al*., 2002). Generally, the antidiabetic property of sweet potato has been attributed to its phytochemical content. Zhao *et al*. (2007) isolated flavone from the leaves of sweet potato and evaluated its effects on different markers of diabetes. There was a significant decrease in the fasting plasma insulin and blood glucose level and significant increase in the insulin sensitive index in non-insulin dependent diabetic rats.

2.1.5.3 Anticancer potential

Extracts from different parts of sweet potato have also been reported to exhibit anticancer and antitumor properties. Sweet potato extract inhibits proliferation and induces apoptosis in prostate cancer cells *in vivo* and *in vitro* (Karna *et al.*, 2011), this activity was attributed to the high polyphenol content of the extracts. Similarly, in a very recent study, purple fleshed sweet potato extracts were found to have inhibitory effect on the growth of MCF-7 (breast cancer) and SNU-1 (gastric cancer) cancer cell lines (Sugata *et al* ., 2015). The therapeutic potential of purple fleshed sweet potato has mostly been attributed to its high anthocyanin content. Anthocyanins or anthocyanin-rich extracts have displayed inhibitory effect on cancer cell growth in various cancer cells (Wang and Stoner, 2008). A group of researchers also reported that purified protein from the storage root of sweet potato promotes dose- and time-dependent inhibition of human colorectal cancer SW480 cell proliferation, migration and invasion (Li *et al.*, 2013).

2.1.5.4 Antiulcer potential

Ulcer is characterized by the shedding of inflamed tissue from the skin or mucous membrane (Hermes *et al.*, 2013). Methanol extract of sweet potato roots showed gastroprotective activity against asprin induced ulcer in Wistar rats in a dose dependent manner (Panda and Sonkamble 2012). The flour of sweet potato tubers potentially inhibited ethanol-induced gastric ulceration by suppressing edema formation and partly protecting gastric mucosa wrinkles (Hermes *et al.*, 2013). In another *in vivo* study, ethanolic extract of sweet potato roots showed antacid-like action against a pylorus ligation and cold restraint stress induced ulcer in animal models (Rengarajan *et al.*, 2012).

2.1.5.5 Effect on cardiovascular system

The oxidation of low-density lipoprotein can cause complications which can result into atherosclerosis leading to cardiovascular disease (Steinberg, 2009). Studies by Nagai *et al.*, (2011) indicated that sweet potato leaf extract was able to suppress low density lipoprotein oxidation *in vitro*

and in human subjects. This suppression was attributed to the antioxidant activity of phytochemicals present in the leaves. Anthocyanin, which is an abundant phytochemical in purple fleshed sweet potato, has been reported to be able to reduce the risk of coronary disease (Mazza, 2007).

2.1.5.6 Effect on immune system

Sweet potato extracts have also been reported to have modulatory effects on the immune system and health. Ethyl acetate fractions of bioactives extracted from two different cultivars of sweet potato exhibited immunomodulatory activities in a cultivar dependent manner in mice splenocytes (Chen *et al.*, 2013). Hanieh *et al.* (2010) reported that dietary supplementation of purple sweet potato improved immune response after immunization in chickens. Similarly, the consumption of purple sweet potato leaves was able to modulate T-lymphocyte functions, lytic activity of natural killer cell and antibody production in a study involving 16 healthy human adults (Chen *et al.*, 2005). Reports of immunomodulatory studies on sweet potato are mostly on purple skinned potato cultivars. It has been postulated that extracts from purple sweet potato improve immune dysfunction possibly by modulating antioxidant defense systems (Kim *et al.*, 2015). A dietary supplement with purple sweet potato extract increased the activity of the antioxidant enzymes, superoxide dismutase and glutathione peroxidase in LP-BM5 murine leukemia virus-induced murine acquired immune deficiency syndrome (Kim *et al.*, 2015). The cooked leaves of sweet potato leaves also showed immunomodulatory effect when consumed by basketball players during a training period. The plasma concentration of polyphenols in the players increased significantly during this period coupled with a significant increase in the cytotoxic activity of nature killer cells, and secretion of interferon (IFN)-γ (Chang *et al.*, 2007).

2.1.5.7 Antimicrobial effects

Although reports of the antimicrobial activities of sweet potato tubers are limited, antimicrobial activity of the leaves has been reported in a number of studies. Acetone and ethanol extracts of sweet potato leaves showed antimicrobial activity against *Salmonella typhimurium* and *Pseudomonous auroginosa* respectively, while n-hexane and ethyl acetate extracts did not show any antimicrobial activity against the said strains (Adsul *et al*., 2012). Mbaeyi-nwa and Emejulu (2013) tested the antimicrobial activity of peptone, water and ethanol extracts of sweet potato leaves against *E. coli*, *S. typhi*, *S. aureus*, *A. niger*, *Penicillium spp*., *P. aeroginosa* and *K. pneumonia*. In their findings, they reported that the water extract exhibited high antimicrobial activity by inhibiting the growth of all the organisms except *E. coli* and *Penicillium spp* at different concentrations of the extracts. Dietary fibre from the root of sweet potato also showed inhibitory effect against the growth of food-borne bacteria (Yoshimoto *et al*., 2011) while antimicrobial film made with sweet potato starch incorporated with varying levels of potassium sorbate or chitosan exhibited inhibitory effects on *E. coli* (Shen *et al*., 2010).

2.1.5.8 Antiinflammatory potential

The potential of purple sweet potato extract to inhibit inflammatory brain diseases by suppressing lipo polysacharide (LPS) induced inflammatory responses have been demonstrated by a number of studies. Pretreatment with purple sweet potato extract was able to inhibit the production of pro-inflammatory molecules in LPS activated BV-2 microglial cells (Kang *et al*., 2014). Furthermore, purple sweet potato colour extract was able to suppress the proinflammatory molecules by inhibition of phosphorylated extracellular signal-regulated kinase (ERK), phosphorylated c-Jun nterminal kinase (JNK) expression and nuclear factor kappa B (NF-kB) activation in a group of LPS-stimulated mice (Wang *et al*. 2010).

NWU LIBRARY

2.1.5.9 Haematological effects

Sweet potato leaf is used in traditional medicine as a remedy for anaemia due to its haematinic effects (Osime *et al.*, 2008). In a study by Montejo and colleagues, sweet potato leaves powder diet increased the packed cell volume, heamoglobin levels and red blood cells in mice (Montejo *et al.*, 2015). Similarly, an earlier study reported a significant increase in packed cell volume, white blood cells and platelets of rabbit fed with sweet potato extract (Osime *et al.*, 2008).

2.1.6 Variation in chemical composition and bioactivities of sweet potato cultivars

The level and activity of phytochemicals in plants have been confirmed by various studies to often vary among cultivars of the same species. Different cultivars of the same plant species adapt very differently to their environment, even though they are native of the same environment. These variations in adaptation may consequently affect the nutritional status and also the level of phytochemicals of closely related genotypes of a species (Kennedy and Burlingame, 2003). For instance, a number of experiments have shown that although most pomegranate varieties have similar composition of phytochemicals, the level or amount of the phytochemicals may depend on the cultivar (Legua *et al.*, 2012 : Tehranifar *et al.*, 2010). Similarly, in guava, the antioxidant activity and phytochemical composition of P. guajava varied significantly depending on the cultivars and pulp colors (Flores *et al.*, 2015).

In sweet potato, variation in phytochemical composition and bioactivities in selected cultivars have also been reported by a number of studies (Teow *et al.*, 2007; Surayia *et al.*, 2008; Ji *et al.*, 2015). The caffeic acid content differs significantly across 24 cultivars of sweet potato (Harrisson *et al.*, 2003), while the total and individual phenolic acid among 6 sweet potato cultivars investigated by Padda and Picha (2007) varied significantly across the cultivars. A recent comparative study by Shekar and colleagues of orange and white fleshed sweet potato revealed increased levels of protein, flavonoids, anthocyanins and carotenoids in orange fleshed sweet potato than in white fleshed sweet

potato (Shekar *et al.*, 2015). They also reported that although differential proteomic analysis indicated several spots common to both cultivars, certain spots were peculiar to either the orange fleshed or the white fleshed sweet potato.

Similarly, in the analysis of four (4) different (purple, red, yellow and white) sweet potato cultivars, the highest antioxidant activity was recorded in the purple variety while other parameters investigated varied significantly across the different cultivars (Ji *et al.*, 2015). Studies have indicated that the antioxidant activity in purple fleshed sweet potato is relatively higher than other varieties of sweet potato (Kim *et al.*, 2011; Park *et al.*, 2014). Different cultivars of sweet potato with varying flesh colors also exhibited varying antimicrobial activities. 'White star', a Pakistani cultivar of sweet potato showed better anti-diabetic potential than 'Beuragard' a US cultivar as shown from the postprandial glucose level and insulin response in diabetic patients (Surayia *et al.*, 2008). Although most reports of the anti-hyperglycemic potential of sweet potato leaves do not specify the variety of the plant used, the antidiabetic activities of sweet potato tuber has mostly been associated with the white skinned variety (Ludvik *et al.*, 2004; Niwa *et al.*, 2011). In general, the orange varieties have high beta-carotene content and the ability to combat vitamin A deficiency. The reports of antiinflammatory and immunomodulatory activities are mostly associated with the purple fleshed sweet potato variety. As in other plants, proper analysis and selection of cultivars with the optimal and desired phytochemical composition would remain important to exploring the medicinal/therapeutic potential of sweet potato.

Table 2.2a: Reports of pharmacological activities of sweet potato

Investigation	Cultivar colour description	Component of sweet potato used	Type of study/subject	Results
Antidiabetic activity of flavone extracted from the leaves leaf (Zhao *et al*., 2007)	N/S	Flavone extracted from the leaf	Non-insulin dependent diabetic rats	Modulate the metabolism and lowers blood glucose level
Antioxidant activities (Teow *et al*., 2007)	Cream, white, orange ,yellow and purple	Hydrophilic and lipophilic fractions of Hexane extract of root tubers	In vitro assays	Highest antioxidant activity recorded in purple fleshed and the lowest in white fleshed varieties
Immunomodulatory effects after immunization in chickens (Hanieh *et al*., 2010)	Purple	Sweet potato powder as dietary supplementation	Chickens	Immune response of chicken after immunization was improved
Wound healing and antiulcer properties (Hermes *et al*., 2013)	White	Tuber flour	Wistar rats	Shows wound healing potential

N/S- not specified

Table 2.2b: Reports of pharmacological activities of sweet potato

Investigation	Cultivar colour description	Component of sweet potato used	Type of study/subject	Results
Effects on level of blood glucose and Hepatic Enzymes (Ogunrinola *et al.*, 2015)	N/S	Aqueous extract of leaf powder	Mice	Lowers blood glucose, reduces the activity of hepatic enzymes
Antihyperglycemic/ antidyslipidemic potential (Pal *et al.*, 2015)	N/S	ethanolic, ethanolic: aqueous and aqueous extracts of leaves	Mice	Improvement in blood glucose profile
Antidiabetic efficacy and hypoglycemic mechanisms (Niwa *et al.*, 2011)	White	Aqueous extract of the whole tuber	Streptozotocin induced-diabetic rats	Increase in serum insulin level and reduced fasting plasma glucose
Antiinflammatory and Anticancer Activities (Sugata *et al.*, 2015)	Purple	Crude anthocyanin extracts	In vitro, cancer cell lines	Inhibit the growth of cancer cells and proinflammatory cytokines

N/S- not specified

Table 2.2c: Reports of pharmacological activities of sweet potato

Investigation	Cultivar colour description	Component of sweet potato used	Type of study/subject	Results
Characterization, antioxidant and antitumor activities (Wu *et al.*, 2015)	Purple	Three polysaccharides extract	Cell line	Inhibit the growth of tumour cells
Immunomodulatory and Antioxidant Effects on Induced Murine Acquired Immune Deficiency Syndrome (Kim *et al.*, 2015)	Purple	Aqueous and ethanol extract of root tubers	Mice	Show potential to improve immune dysfunction by modulating antioxidant defense systems.
Effect on apoptosis and Inflammatory Adipokine Expression (Lee *et al.*, 2015)	Purple	Hot water extract of the leaves	3T3-L1 cell line	Induced apoptosis and downregulated inflammation-associated genes
Antifungal Activities of Anthocyanins from Sweet Potato (Wen *et al.*, 2016)	Purple	Anthocyanin extract with preservatives	*In vitro* assay	Enhance antifungal activity

N/S- not specified

2.2 TYPE 2 DIABETES MELLITUS

Type 2 diabetes is a metabolic disease, which is characterized by hyperglycemia, a condition resulting from insulin resistance, impaired insulin secretion or both. It is the most common type of diabetes as it represents 90% of diabetes cases (Hassan, 2013). Type 2 diabetes is a serious global health concern with startling increasing incidences especially in developing countries (Wu *et al.*, 2014). It is a multipathogenic disease and hence, different approaches and strategies are usually employed in its management. Exercise, diet changes and drug therapy are common approaches to achieve desirable glycemic control in type 2 diabetes (Eriksson and Lindgarde, 1991; Boule *et al.*, 2001; Padwal *et al.*, 2005). Insulin resistance and hyperglycemia are major hallmarks of type 2 diabetes, therefore, drug therapies for type 2 diabetes are usually targeted at improving insulin sensitivity and maintenance of glycemic control. These can be achieved through mechanisms such as the modulation of genes involved in glucose and fatty acid metabolism, amelioration of oxidative stress and inhibition of carbohydrates metabolizing enzymes. Scientific evaluation of different herbs, wild and edible plants has demonstrated the anti-hyperglycemic effects of phytochemicals (Gaikwad *et al.*, 2014). Plants and their products have become promising alternatives in the management and treatment of type 2 diabetes.

2.2.1 Insulin resistance in type 2 diabetes

Insulin resistance occurs when there is a diminished metabolic response of target cells of the body to insulin action (Bugianesi *et al.*, 2005). It is an important and powerful factor in type 2 diabetes which makes it a therapeutic target in the management of the disease (Taylor, 2012). Type 2 diabetes is characterized by insulin resistance in various body tissues, especially in the skeletal muscle which is the main site for insulin-stimulated glucose utilization (Sears and Perry, 2015). In skeletal muscle, insulin resistance is attributable to impaired glucose and/or fatty acid metabolism (Defronzo and Tripathy, 2009).

2.2.2 Glucose and fatty acid metabolism in insulin resistance

Glucose is an essential metabolic substrate of all mammalian cells. It is the major carbohydrate utilized for energy production and many other anabolic requirements in mammals (Szablewski, 2011). However, low levels of glucose in the blood can lead to unconsciousness or seizures (Halawa *et al*., 2015) while a persistent hyperglycemic condition can result into complications such as nephropathy, neuropathy and peripheral vascular diseases (Buren, 2003). Glucose concentration in the blood depends on the rate at which glucose enters the blood against the rate at which it is removed from the circulation (Arnoff *et al*., 2004). Therefore, accurate matching of glucose production and utilization is needed to maintain a normal plasma glucose concentration (Giugliano *et al*., 2008). This process is called glucose homeostasis and it is achieved through finely hormone regulation of glucose uptake from carbohydrate diet, peripheral glucose utilization and hepatic glucose production (Szablewski, 2011). Skeletal muscle is a primary target in the management of insulin resistance because it is the major site for glucose uptake in the body (Sears and Perry, 2015). In the skeletal muscle cells, insulin-stimulated transport of glucose relies on a number of signaling components which results in the translocation of the glucose transporter 4 (GLUT4) to the cell surface (Pessin and Saltiel, 2000). In essence, the action of insulin on muscle cells for glucose uptake depends on the induced translocation of GLUT4. GLUT4 is a transmembrane protein that allows the transport of peripheral blood glucose across the plasma membrane into the cell through facilitated diffusion (Huang and Czech, 2007). The GLUT4 gene promoter has a binding region for MEF2A transcription factor to facilitate GLUT4 expression (Mcgee *et al*., 2006). Thus, MEF2A also functions as a major transcriptional regulator of glucose uptake metabolism. Another important transcription factor in glucose transport is the NRF1 gene which is involved in the transcriptional activation of MEF2A through peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) (Ramachandran *et al*., 2008; Ferreira *et al*., 2014; Wu *et al*., 2016).

Other than glucose, fatty acids which are essential components of membrane lipids and a major source of energy also play an important role in the pathogenesis of metabolic diseases including type 2 diabetes (Wakil and Abu-Elheiga, 2009). Acetyl-CoA carboxylases (ACC) are important regulatory enzymes involved in the regulation of fatty acid synthesis and oxidation in response to dietary changes (Fillmore *et al.*, 2011). The two isoforms of ACC; ACC1 and ACC2 provide the substrate (malonCoA) for the synthesis and oxidation of fatty acid respectively; hence they both play important roles in fatty acid metabolism. Another important component of fatty acid metabolism is carnitine palmitoyltransferase I (CPT1), a mitochondrial transmembrane enzyme involved in the facilitation of long-chain fatty acid entry into the mitochondria for beta oxidation (Bruce *et al.*, 2009; Chen *et al*, 2016). In the skeletal muscle, ACC2 acts mainly by inhibiting the activity of CPT1 thereby prevent the oxidation of fatty acid. Lipid accumulation, which results from a mismatch between fatty acid uptake and oxidation, has been implicated in the pathology of insulin resistance in type 2 diabetes (Zhang *et al.*, 2010). Wakil and Abu-Elheiga (2009) demonstrated that lack of ACC2 will lead to increased fatty acid oxidation which cause a decreased level of fatty acid levels and the up-regulation of insulin signaling (Figure 2.2). The improved insulin signaling will lead to increased translocation of GLUT4 to the plasma membrane and ultimately increased glucose uptake and lowered blood glucose level. On the other hand, increased oxidation of fatty acid at the expense of glucose oxidation and utilization has also been linked to the development of insulin resistance especially in the heart (Lopaschuk, 2016). The specific role of fatty acid metabolism in the development of insulin resistance still remains controversial and unclear (Zhang *et al.*, 2010).

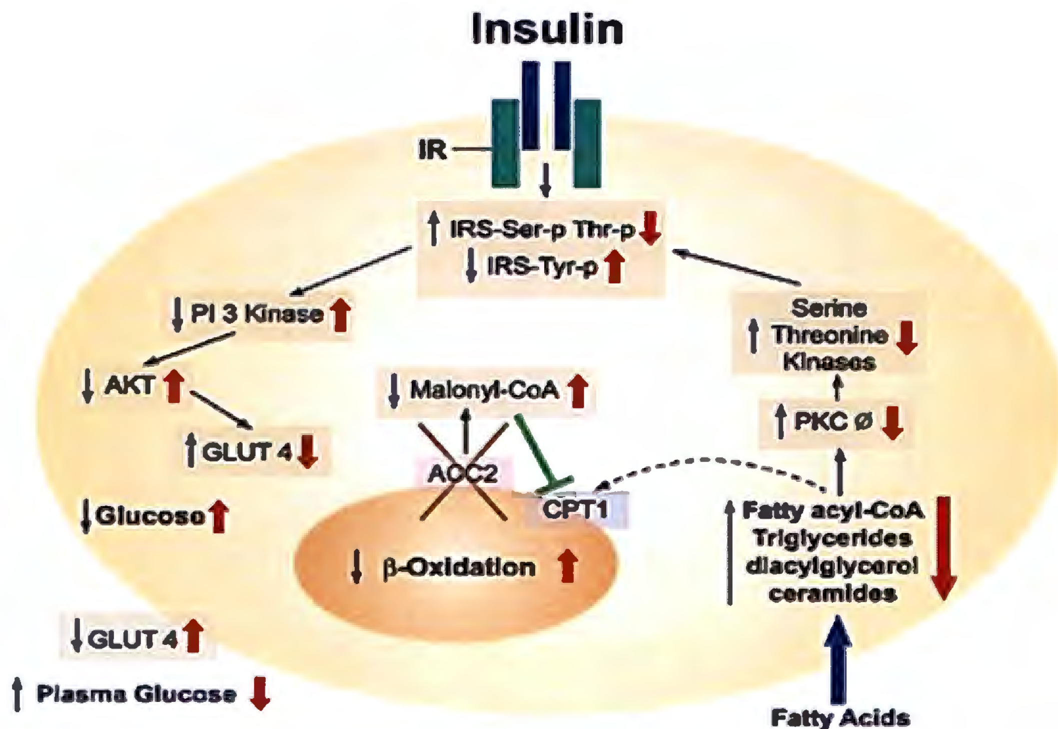


Figure 2.2: Fatty acid oxidation and glucose uptake in peripheral cells such as the skeletal muscle. High level of fatty acyl-CoA and triglyceride (blue arrows) causes the activation of PKC Ø leading to increasing serine and threonine phosphorylation of Insulin receptor substrates (IRS1 and IRS2). These substrates produce IRS-Ser-p Thr-p, which causes the reduction in the activity of PI 3 kinase, down-regulates AKT, leading to decrease in the translocation of GLUT4 to the plasma membrane and in glucose uptake. The knockout of ACC2 will result into a continuous oxidation of fatty acid and hence a lower level of fatty acyl CoA which will ultimately lead to increased translocation of GLUT4 and increased glucose uptake (orange arrows) (Wakil and Abu-Elheiga, 2009) .

2.2.3 Oxidative stress in insulin resistance and type 2 diabetes mellitus

Oxidative stress is one of the factors that have been associated with the pathogenesis of type 2 diabetes and its complications. Oxidative stress occurs when the production of oxidants and its effects exceeds the ability of the body antioxidant defense system to counteract the resulting damage (Sies, 1991; Betteridge, 2000). Several studies have demonstrated increased oxidative stress/damage and reduced antioxidant defense system in type 2 diabetic conditions (Park *et al.*, 2009; Tangvarasittichai, 2015). This means that oxidative stress contributes to the development of insulin resistance in the prediabetes stage as well as its progression to type 2 diabetes and its complications. Excess free radicals such as reactive oxygen species (ROS) production and the inability of the antioxidant defense system to combat the effect ultimately lead to a condition of oxidative stress (Poljsak, 2013; Shen and Liu, 2006). Oxidative stress has a damaging effect on insulin signaling by initiating a sequence of pathways involving a family of serine/threonine kinases and thus causes the suppression of insulin sensitivity (Tigani *et al.*, 2011; Rains and Jain, 2011). The overproduction of hydrogen peroxide and superoxide ion in the mitochondria and the increased activation of cellular NADPH oxidase through angiotensin II receptors are some of the mechanisms that have been implicated in the generation of excess ROS (Henriksen *et al.*, 2011). This overproduction of ROS occurs through pathways such as the polyol pathway and protein kinase C (PKC) activation (Tangaracittichai, 2015).

The polyol pathway is a two-step metabolic pathway that has been implicated in the development of complications such as diabetic neuropathy (Oates, 2002), diabetic nephropathy (Dunlop, 2000) and diabetic retinopathy (Lorenzi, 2007; Mathebula, 2015). The pathway is activated when there is high level of glucose in the cell. Aldose reductase reduces the excess glucose to sorbitol using up NADPH in the process and oxidizing it to $NADP^+$, sorbitol is then oxidized to fructose by the enzyme sorbitol dehydrogenase (Mathebula, 2015). The decrease in the available NADPH in turn reduces the

synthesis of glutathione reductase. Glutathione reductase is major cellular antioxidant enzyme which catalyses the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), an important molecule in the suppression of oxidative stress within the cell (Deponte, 2013). Another source of oxidative stress from this pathway is the oxidation of sorbitol to fructose, which involves the conversion of NAD^+ to NADH, the substrate for NADH oxidase which is involved in the generation of reactive oxygen species (ROS) (Mathebula, 2015).

The hexosamine pathway, where glutamine fructose-6-phosphate amidotransferase (GFAT) catalyzed the conversion of fructose-6-phosphate to glucosamine-6-phosphate (Buse, 2006) and advanced glycation end-products (AGEs) formation are other mechanisms associated with oxidative stress generation in type 2 diabetes (Tangaracittichai, 2015). Figure 2.3 summarises the different sources of ROS production in Type 2 diabetes.

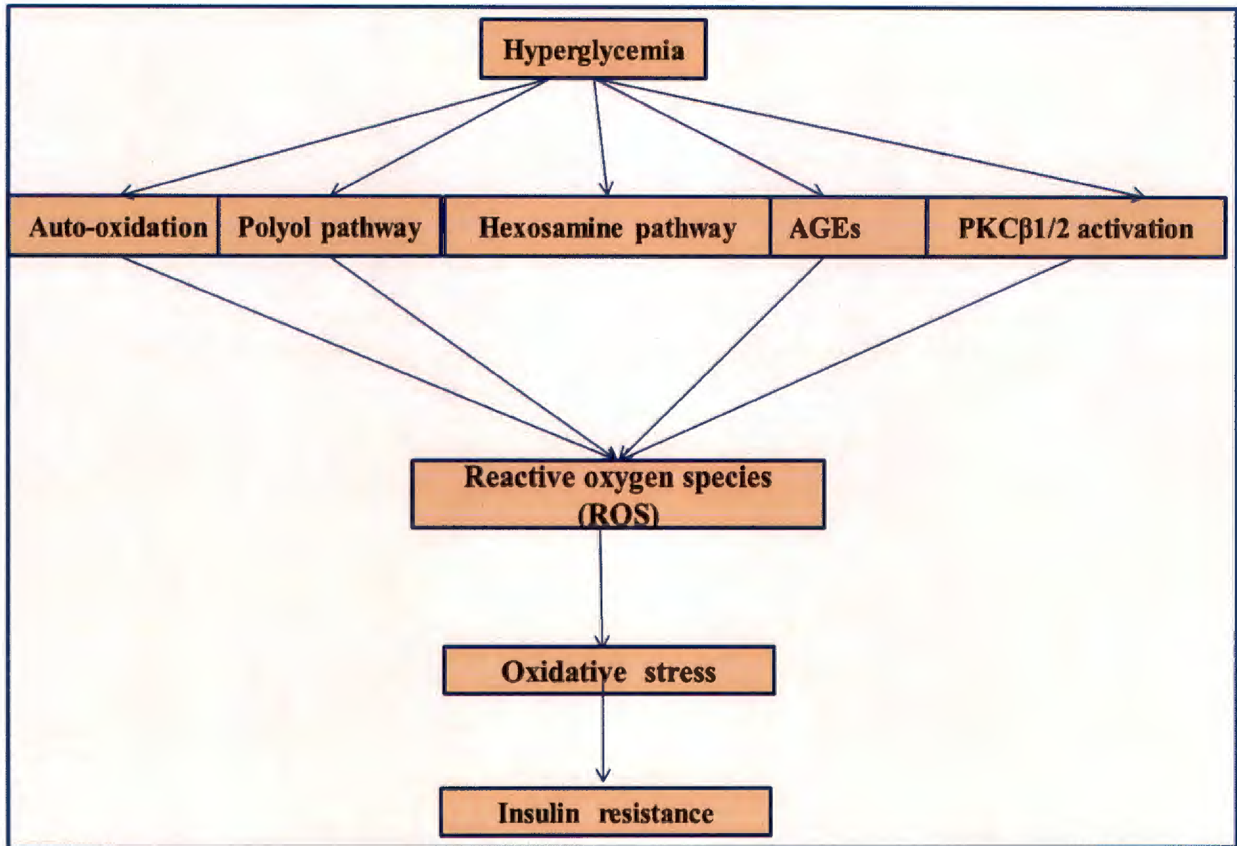


Figure 2.3: Different sources of ROS production in insulin resistance in Type 2 diabetes. AGEs, Advanced glycation end products; PKC, Protein kinase C (Adapated from Tangvarasittichai, 2015)

2.2.4 Antioxidant defense system

Cells have developed highly complex antioxidant systems, which work together to protect the body against oxidative damage (Bhajaj and Khan, 2012). The body antioxidant defense system is made up of different specific enzymes, metal binding proteins, vitamins and some low molecular weight compounds such as glutathione, most of which can be measured in the body to assess the level of oxidative stress in the body (Gawlik *et al.*, 2015). Enzymatic antioxidants such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) and non-enzymatic ones such as reduced glutathione are important antioxidants in the body defense system (Jeeva *et al.*, 2015).

Glutathione peroxidase is a group of selenium-containing antioxidant enzymes that catalyse the reduction of H_2O_2 and lipid peroxides to water and lipid alcohols, respectively and in the process oxidize glutathione to glutathione disulfide (Tabet and Touyz, 2007). The activity of glutathione peroxidase prevents the cells from oxidative stress and damage by inhibiting lipid peroxidation and converting lipid peroxides to lipid alcohols.

Lipid peroxidation is the oxidative degradation of lipids, lipopoteins and other molecules that contain lipids in oxidative stress conditions (Nikki, 2008). The extent of lipid peroxidation is also indicative of the antioxidant status of the cell. The level of malonaldehyde, a toxic byproduct of lipid peroxidation is indicative of the extent of lipid peroxidation and is usually measured to determine intracellular antioxidant status (Ayala *et al.*, 2014).

Catalase catalyzes the reduction of hydrogen peroxide to water and molecular oxygen, thereby preventing the accumulation of hydroxyl radical (El, 2012). Superoxide dismutase is another important antioxidant enzyme that catalyzes the removal of superoxide free radicals by converting the superoxide radical into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (Che *et al.*, 2016). The levels of activity and expression of these antioxidant enzymes are important indicators of intracellular antioxidant status. Significant changes have been reported in the expression and

activities of GPx, CAT and SOD in type 2 diabetic conditions when compared with non-diabetic condition (Ahmed *et al.*, 2006; Aouacheri *et al.*, 2015). Antioxidant enzymes play important roles in the scavenging of free radicals and the maintenance of redox status in the cell.

Glutathione (GSH) is a tripeptide which can be found in every part of the body of living organisms especially in the liver, lungs and the intestinal tract (Lushchak, 2012). It is an important and major component of the cellular antioxidant defense system. GSH is synthesized in the cytosol and thereafter distributed to other intracellular organelles (Lu *et al.*, 2009). GSH can directly scavenge free radicals, act as a substrate for glutathione peroxidase and as co-factor for various enzymes to maintain cellular redox status (Maritim *et al.*, 2003). A reduced level of glutathione has been associated with insulin resistance and type 2 diabetes. Sekhar *et al.* (2011) reported a reduced glutathione level in adult patients with uncontrolled type 2 diabetes when compared with non-diabetic subjects. Similarly, the study of Kalkan and Suher (2013) demonstrated a reduced GSH level in patients with onset of type 2 diabetes when compared with healthy subjects. The reduced level of glutathione in diabetic conditions can be attributed to increased oxidative stress and consequently the increased depletion of intracellular GSH to scavenge free radicals and maintain redox status within the cell (Calabrese *et al.*, 2012).

2.2.5 The role of carbohydrate metabolizing enzymes in type 2 diabetes

Carbohydrate metabolizing enzymes are biological catalysts that are important in the breakdown and digestion of carbohydrate (Sinha *et al*, 2015). The inhibition of carbohydrate metabolizing enzymes is one of the strategies that have been employed in the management of postprandial hyperglycemia in type 2 diabetes. This inhibition is achieved through the control of starch breakdown and absorption of intestinal glucose (Kwon *et al.*, 2008). Improper digestion and absorption of carbohydrate have been implicated in the pathology of obesity and type 2 diabetes (Laar *et al.*, 2008). Thus, a therapeutic approach which involves the inhibition of carbohydrate hydrolyzing enzymes can be

employed in the management of type 2 diabetes. Alpha glucosidase, alpha amylase, sucrase and maltase are important enzymes involved in the digestion of carbohydrates. Alpha-glucosidase is an enzyme in the brush border of the small intestine which breaks down starch and disaccharides to glucose. Alpha-amylase catalyses the hydrolysis of internal α-1, 4-glycosidic linkages in starch to produce glucose and maltose (Sundarram and Murthy, 2014). It exists in the saliva (as ptyalin) and also in the pancreatic juice of the duodenum and the small intestine (Sinha *et al.*, 2015). Alpha-glucosidase and alpha-amylase are the most important targets in the development of therapies for the management of type 2 diabetes. Acarbose is an effective drug that has been used to maintain postprandial blood glucose level. It is an inhibitor of alpha glucosidase in the small intestine and pancreatic alpha amylase enzymes (Ag, 1994; Fujisawa *et al.*, 2005). Acarbose acts by slowing down the rate of digestion of carbohydrates resulting into less glucose level in the blood and hence less absorption of glucose in to the body cells (Ag, 1994) .Other drugs that are being used as inhibitor of carbohydrate metabolizing enzymes are migitol and voglibose (Cai *et al.*, 2013).

The inhibitory actions of natural products and functional foods on alpha glucosidase and alpha amylase have been reported by a number of studies. Flavonoids which are dietary polyphenols found in most fruits and vegetables are able to potently inhibit yeast alpha glucosidase and porcine alpha-amylase enzymes (Tadera, 2006; Lo Piparo 2008). Yilmazer-Musa *et al.* (2012) carried out a study on tea and grape seed extracts. Their results showed that grape seed extract showed stronger inhibition against alpha-glucosidase activity and as much inhibition against alpha-amylase when compared with the standard drug, acarbose. Although the tea extract did not strongly inhibit alpha amylase, it was able to potently inhibit alpha glucosidase. The ability of plants to inhibit carbohydrate enzymes have been linked to the action of either a singular compound or the synergistic action of two or more phytochemicals present in the plant (Yin *et al.*, 2014). Plants and their products can be utilized as inhibitors of carbohydrate metabolizing enzymes in the management of postprandial blood glucose in type 2 diabetes.

2.2.6 Phytotherapy and functional foods in the management of type 2 diabetes

Plants contain a wide range of chemicals which are capable of interfering with the body metabolic process enough to result into the treatment or alleviation of metabolic diseases. Plants have always been part of the traditional means of treating and managing different diseases including type 2 diabetes. They are sources of phytochemicals which can act as insulin mimetic, insulin secreting and insulin sensitizing agents in type 2 diabetic conditions (Kazeem and Davies, 2016). Phytochemicals can exert their antidiabetic properties through various strategies such as their ability to ameliorate oxidative stress (Sabu *et al.*, 2002; Srinivasan and Pari, 2012), inhibit carbohydrate metabolizing enzymes (Etxeberria *et al.*, 2012; Adefegha and Oboh, 2012) or regulate genes and transcription factors involved in type 2 diabetes (Wang *et al.*, 2011; Anuradha, 2013). Wild plants, herbs and food crops such as fruits, vegetables, roots and tuber crops are all forms of plants that have been explored as herbal remedy for type 2 diabetes.

Bitter melon (*Momordica charantia*) is a vegetable plant that has been used in Ayurveda medicine to treat various diseases and different parts of the plant have been studied extensively for its antidiabetic properties. It ameliorates insulin resistance in high fat fed diet (Shih *et al.*, 2014), lowers blood sugar in streptozotocin induced diabetic rats (Wehash *et al.*, 2012) and in diabetic patients (Efird *et al.*, 2014). A charantin rich extract of the plant also promotes insulin sensitivity by upregulating the expression of GLUT4 and IRS1 in skeletal muscle and liver of type 2 diabetic mice respectively (Wang *et al.*, 2014). Teas such as green tea and rooibos are also some plants that have shown antidiabetic potential in type 2 diabetic models. For example, green tea lowered blood glucose level and improved glucose tolerance in a type 2 diabetic mouse (Tang *et al.*, 2013). Furthermore, a study by Kamakura *et al.* (2015) investigating the antidiabetic activity of unfermented green rooibos extract (GRE) found that the extract acted through different mechanisms. These mechanisms include the enhancement of glucose uptake by the induction of phosphorylation of 5′-adenosine

monophosphate-activated protein kinase (AMPK) and promotion of phosphorylation of Akt in L6 myotubes by GRE. In the same study, GRE also suppressed the increase in ROS levels caused by advanced glycation end products (AGEs) in pancreatic β-cells. The antidiabetic properties of these plants have been largely attributed to their phytochemical constituents. Generally, the most prevalent group of beneficial phytochemicals in plants is the polyphenols (Kiero *et al.*, 2013). Polyphenols have therapeutic potential in various diseases which have been largely attributed to their antioxidant capacity (Pajak *et al.*, 2014). Sweet potato, particularly the white fleshed varieties have been reported to have antidiabetic potential (Kusano and Abe, 2000; Ludvik *et al*, 2002). However, orange fleshed varieties which have been sometimes found to contain more polyphenols than the white varieties have not been explored for their antidiabetic properties. Soybean (Veleso *et al.*, 2008), guava (Guo *et al.*, 2013; Liu *et al.*, 2013) and pumpkin (Chang *et al.*, 2014) are some other food crops that have demonstrated their therapeutic potentials in type 2 diabetic models. Therefore, beyond their traditional nutritional properties, food crops can be harnessed in the development of herbal drugs for type 2 diabetes.

2.2.7 C2C12 skeletal muscle cell as an *in vitro* model of insulin resistance

Skeletal muscle is one of the major insulin-dependent tissues responsible for maintenance of glucose homeostasis in the body (Yap *et al.*, 2007). *In vitro* models are widely used in the preliminary evaluation of compounds because they offer a controllable environment in terms of factors such as pH, temperature and time (Joshi *et al.*, 2011). They also allow for specific cellular and molecular studies even within a limited period of time. One of the commonly used *in vitro* models of skeletal muscle is C2C12 cells. C2C12 cells are immortalized mouse cell lines which have the characteristics of skeletal muscle cells and are capable of rapid proliferation into myoblasts under high serum conditions. Under low or no serum conditions, the myoblasts differentiate to form multinucleated myotubes (Burattin *et al.*, 2004). C2C12 cells are widely used for the detailed studies on the

mechanisms of insulin-dependent glucose uptake and transport mediated by GLUT 4 in muscle cells (Nedachi and Kanzaki 2006; Park *et al*., 2007; Ijuin and Takenawa, 2012; Zhang *et al*., 2016). They serve as important screening tools for the assesment of antidiabetic properties and mechanisms of actions of potential therapeutic agents prior to an appropriate *in vivo* study (Dsouza and Lakshmidevi, 2015). Therefore, C2C12 cells were chosen as the *in vitro* model of insulin resistance in this study to evaluate the antidiabetic effects and mechanisms of action of sweet potato extracts.

CHAPTER THREE

ANALYSIS OF NUTRIENTS AND CHEMICAL CONSTITUENTS OF THE TUBERS OF SEVEN SOUTH AFRICAN GROWN CULTIVARS OF SWEET POTATO (*IPOMOEA BATATAS L.*).

ABSTRACT

Nutritional and chemical composition of the tubers of seven different sweet potato cultivars cultivated in South Africa was determined. Four white-fleshed cultivars (Blesbok, Ndou, Monate, Mvuvhelo) and three orange-fleshed cultivars (Bophelo, Impilo, 199062.1) were used in this study. Protein, ash, crude fibre analyses were done using standard AOAC procedures. Fatty acids and sugar contents were carried out using GC-MS. Carbohydrate and energy values were determined using standard methods. Detection of metabolites in the aqueous and aqueous methanol extracts of the tubers was determined using GCMS. The results showed that Bophelo had the highest protein content (12.00%) with Blesbok having the lowest protein content (4.56%). The study also revealed that Impilo had the highest fibre (7.11%) and total sugar (22.21 %) contents which were significantly higher than those of Bophelo, Ndou, Monate, Mvuvhelo and 199062.1. The highest level of ash content which is an indication of minerals was found in 199062.1 (5.81 %) and lowest was in Monate (4.50 %). The study indicated that Bophelo had the highest total fat content (0.650 %) with saturated fat (0.270 %) mono-saturated fat (0.050 %) and poly-saturated fat (0.270 %) while the lowest was Impilo (0.380 %) with saturated fat (0.225 %) mono-saturated fat (0.020 %) and poly-saturated fat (0.135 %). Different fatty acids such as palmitic acid (C16), stearic acid (C18), arachidic acid (C20), oleic acid (C18:1), and linoleic acid (C18:2) were also found in the sweet potato cultivars. The highest total carbohydrate content was found in Blesbok (76.72 %) and was significantly higher than Bophelo (69.42 %), Mvuvhelo (74. 51 %), Impilo (75.49 %) and 199062.1 (68.65 %). However, Ndou and Monate had comparable carbohydrate contents with Blesbok. The different sugar components i.e.

fructose, glucose, maltose, sucrose were also present in the sweet potato cultivars at varying concentrations. To mention a few, different phytochemicals such as glycolic acid, propanoic acid, 2-[(trimethylsilyl)oxy]-trimethylsilyl ester, pentasiloxane, dodecamethyl, glycerol, tris(trimethylsilyl) ether were detected in the sweet potato cultivars. In conclusion, this study demonstrates significant variation in the chemical composition of the different cultivars. Knowledge on the nutritional qualities of these cultivars will be useful for informed dietary advice and consumption. Breeders can also harness the data and information on the varying composition of the different cultivars for the development of improved sweet potato cultivars.

3.1 INTRODUCTION

Malnutrition contributes severely to human suffering and seriously affects the socio-economic development of a nation due to a work force that is mentally and physically stunted and may have a reduction in work strength (Dhakar *et al.*, 2011). An important approach against malnutrition is the promotion of underutilized traditional crops for food security and nutrition (Mayes *et al.*, 2011; Chivenge *et al.*, 2015). There is a need for greater efforts to increase the supply of healthy food at rate comparable to population growth or even faster, otherwise many people will suffer from malnutrition (Al-Fargaet *et al.*, 2016). Starchy root and tuber crops are next in terms of importance to cereals as global sources of carbohydrates and are vital source of animal feed and processed products for human consumption and industrial use (Chandrasekara and Kumar, 2016). Plants can also serve as medicinal source as the use of plants and their products for different purposes has been with man from the beginning (Agbafor *et al.*, 2015).

Sweet potato (*Ipomoea batatas*) is an important crop present in different areas of the world, being produced in more than 100 countries (Alan *et al.*, 2016). The crop is produced mainly in Asia which account for up to 76.1% of world production in 2013, followed by the African continent (19.5%) (FAO, 2015; Hue and Low MY, 2015). It is rated as the seventh most important food crop across the world (Shekhar *et al.*, 2015). Maximum yields and better quality

roots are obtained on a well-drained, sandy or silt loam soil and requires both warm days and nights for optimum growth and root development (Ayeleso *et al.*, 2016). Sweet potato is one of the food security crops that can help in the alleviation of poverty among rural dwellers through better processing methods and food diversification (Omodamiro *et al.*, 2013). Sweet potato, when compared with the other tubers, contains an average amount of proteins and carbohydrates mainly starch, with some free sugars which give the tuber its sweet taste (Rose and Vasanthakaalam, 2011). Different parts of the plants are known to be a useful source of unique natural products which can be used as therapeutic agent against various diseases and in making industrial products (Mohanraj and Sivasankar, 2014).

It has been reported that phytochemicals in sweet potato possess multifaceted actions such as antioxidant, antimutagenic, anti-inflammatory, antimicrobial and anticarcinogenesis effects and as such, are vital for several health-promoting functions in humans (Konczak-Islam *et al.*, 2003; Ayeleso *et al.*, 2016). The efficient use of sweet potato chiefly determines the varieties that will be grown, whether as food either directly or in processed forms as feed component or as sources of industrial starch (Omodamiro *et al.*, 2013). In this study, tubers of seven different sweet potato cultivars grown under the same conditions in North-West Province of South Africa were collected and a comparative study on their nutrient and chemical composition was done. Results of this study will contribute to the provision of an opportunity for the identification of the sweet potato cultivars that can provide healthier diet for South Africans and the world at large. It will further create an avenue for breeding of sweet potato cultivars with high nutritional qualities and also contribute to improvement of local level food security and potential for industrial use of the different cultivars.

3.2 MATERIALS AND METHODS

3.2.1 Preparation of tubers for analyses

Sweet potato cultivars (Table 3.1) were obtained from the Agricultural Research Council (ARC), South Africa through the Department of Crop Science, North-West University, Mafikeng campus, South Africa. Three tubers of each cultivar were collected fresh from the university farm. They were rinsed and washed thoroughly with running water. They were cut into thin chips before being air dried at room temperature ($25 \pm 2^{o}C$) in the laboratory. Air dried samples were ground into fine powder (about 500µm mesh size) and packed in an air tight container. They were labeled accordingly and stored at $4^{o}C$ for further use.

Table 3.1: List of selected South African grown sweet potato cultivars

Cultivars	Skin colour	Flesh colour
Blesbok	Purple	White
Bophelo	orange	Dark orange
Ndou	Cream	Cream
Monate	Cream	Cream
Mvuvhelo	Cream	Cream
Impilo	deep orange	Light orange
199062-1	Cream	Light orange

NWU
LIBRARY

3.2.2 Determination of Protein Content

Samples were analysed using the TruSpec-N Leco Protein Analyser according to manufacturer's instruction manual. They were homogenised prior to analysis by using a blender. The regulator pressures on each of the gases were as follows; oxygen and helium are at 241.3 kPa. and compressed/pneumatic air at 275.8 kPa. Leco TruSpec application was switched on, Furnace and Afterburner temperatures were set to 950°C and 850°C respectively. Approximately 100 – 200 mg of the sample was weighed into tin foil. Five blanks. EDTA standard, ProNutro (original) control. and samples in duplicate were analysed. Controls were run after every ten sample runs. The percentage nitrogen was calculated by comparison of peak areas of nitrogen in samples with those of standards. The percentage protein content was calculated as described below:

$\%\,Protein = \%\,Nitrogen\ x\ Protein\ factor$ (6.25) (Van Gelder, 1981)

3.2.3 Determination of Fiber Content

Fibre content determination was done by the method of Association of Official Analytical Chemists (AOAC) (2003a). A homogenous sample (1 g) was quantitatively weighed into beakers. 2-(N-morpholino) ethanesulfonic acid (MES) - Tris buffer solution (40 ml) was added to each beaker. A 50 μl heat stable amylase solution was added while stirring at low speed and covered with an aluminium foil. The samples were then placed in a shaking waterbath at 95-100°C for 35 minutes with continuous agitation. They were removed from hot water and cooled to 60°C. A100 μl protease solution was added to each sample and incubated at 60°C. with continuous agitation for 30 min. After 30 minutes. 0.561 N HCl solution (5 ml) was dispensed into sample while stirring. The pH was adjusted to 4.1 – 4.8 with additional 5% NaOH solution or 5% HCl solution as necessary. A 200 μl Amyloglucosidase solution was then added while stirring on a magnetic stirrer and further incubated in shaking water bath at 60°C for 30 min. To each sample. 225 ml 95% EtOH preheated to 60°C was added to allow precipitation to form at

room temp for 1 hr. The precipitated enzyme digest was filtered through a weighed crucible with celite. The samples were quantitatively transferred by rinsing with 78% ethanol, 95% ethanol and acetone. The crucibles containing residues were dried overnight for 5 hrs at 105°C in air convection oven. The crucibles were cooled in a desiccator and weighed to obtain residue weight.

The percentage of fibre content was calculated using the following formula:

$$\text{Dietary fibre \%} = (((\text{R1} + \text{R2})/2 - p - A - B)/(M1 + M2))/2 \times 100$$

where:

R1 = residue weight 1 from m1; R2 = residue weight 2 from m2

M1 = sample weight 1; M2 = sample weight 2

A = ash weight from R1, p = protein weight from R2

B = blank

$$\text{BLANK, B} = \frac{\text{BR1+BR2}}{2} - BP - BA \ldots$$

where: BR = blank residue

BP = blank protein BR1

BA = blank ash from BR2

3.2.4 Determination of Ash Content

Ash content was determined according to the method of AOAC (2005b). Five grams (5g) of samples, taken in triplicates, were weighed into an empty preweighed crucible and carefully charred over an open flame in the fume cupboard until sample was black and smoke free. The muffle furnace was pre-heated to 500°C before putting the samples and ashing was done overnight. The furnace was turned off to allow cooling before samples removal. The samples were desiccated prior to weighing. The ash content was calculated as described below:

$$\% \text{Ash} = ((\text{W3} - \text{W1}))/(\text{W2} - \text{W1}) \text{X } 100$$

W1= Weight of crucible soon after reaching room temperature

W2= Weight of crucible +sample before ashing

W3= Weight of crucible +sample after ashing

3.2.5 Determination of fatty acid content

Determination of fatty acids was carried out by the modified method of AOAC (2005c). After homogenization of the samples, 1g of samples were weighed into digestion tubes and 100 mg pyrogallic acid was added followed by 2 ml of undecanoic acid (internal standard) solution, 2 ml of ethanol and 10 ml of 32% hydrochloric acid. The tubes were placed in the water bath at 75°C with gently shaking for 40 min. The fatty acids were extracted by diethyl ether and petroleum ether. The organic phase was dried and the residue was methylated using 2 ml of sulphuric acid/methanol reagent and 1 ml of toluene at 100°C for 5 min. After cooling to room temperature, 5 ml distilled water and 1 ml of hexane were added and the hexane solution was then dried with anhydrous sodium sulphate and transferred into a vial for gas chromatographic analyses. Fatty acid methyl esters (FAMEs) were quantitatively measured by Gas Chromatography Flame Ionization Detector (GCFID). Fatty acids are identified by comparing their retention times to the retention times of the suitable standard.

3.2.6 Determination of Sugar Content (mono and disaccharides)

Sugar determination was by the method of AOAC (2003d). Sugar was extracted from the sample by weighing 5g of grounded sample (W1) into a 250 ml beaker and 100 ml of 50% ethanol was added into the beaker. The weight of the beaker with the content was taken (W2) then, placed in a water bath at 85°C for 25 minutes and stirred occasionally at intervals of 10 min to break-up or dissolve the sample. It was cooled at room temperature and 95% ethanol was added to original weight, W2. The sample was filtered through a 0.45 µm nylon syringe filter in vial and injected into an HPLC (Agilent 1100) equipped with a RI detector. Column used was Zorbax

carbohydrate column. The mobile phase was a mixture of acetonitrile and water (80:20, v/v), the flow rate was 1.5 ml/min.

3.2.7 Determination of Carbohydrate Content and energy level

The carbohydrate contents and energy level were determined by using the formulas;

$$\% \, carbohydrate = 100 - (\% \, moisture + \% \, ash + \% \, crude \, fibre + \% \, crude \, protein + \% \, fat)$$

$$Energy\left(\frac{kJ}{100g}\right) = 37(\%Fat) + 17(\%Protein + \% \, Carbohydrate) + 8(\%Total \, Dietary \, Fibre)$$

3.2.8 Metabolites detection with GCMS

Preparation of crude aqueous (AQ) and aqueous/methanolic (1:1) (AQ-ME) extracts of the samples for the detection of metabolites were done by soaking 5g of the powder in 100ml of the respective solvents for 24 hours at room temperature with constant shaking. Extracts were recovered through lyophilization and evaporation using a freeze dryer (Alpha 1-4 LSC Plus) and a rotary evaporator (RE-52A) respectively. Dried extracts were stored in the dark at -20°C. The aqueous and aqueous-methanol extracts of the samples were re-dissolved at a concentration of 10mg/ml in vials. 50ppm 3-Phenylbutyric acid was added to each sample as internal standard. The samples were dried under liquid nitrogen in a solvent evaporator machine. Derivatization was done by the addition of 50 µl of oximation reagent (20mg/ml methoxyamine in pyridine) to the dried samples. The mixtures were vortexed for one minute to dissolve the dried compounds. Silylation was carried out by the addition 50µl of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% (TMCS) to the oximated samples. Samples were transferred into micro-vials for GC-MS analysis.

3.2.9 Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using Graph Prism 6 and compared by the Tukeys range test. Differences were considered significant at $p<0.05$.

3.3 RESULTS

3.3.1 Total protein content

As shown in figure 3.1, Bophelo tubers had the highest protein content (12.00%), followed by 199062.1 (11.25%). The levels of protein content in other tubers were as follows: Blesbok (4.56%), Ndou (5.69%), Monate (6.31%), Mvuvhelo (6.38%) and Impilo (5.19%). The total protein content in Bophelo was significantly higher when compared with the other cultivars at $P<0.05$.

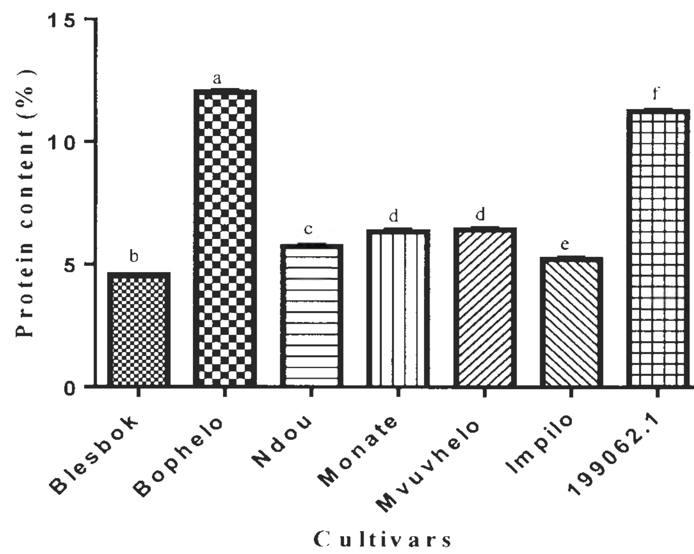


Figure 3.1: Protein contents of tubers of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). Same letters indicate non-significant difference while different letters indicate significant difference at $p<0.05$.

3.3.2 Total fibre content

The result as shown in figure 3.2 indicates that Impilo tubers had the highest fibre content (7.11%), followed by Mvuvhelo (6.66%). The level of fibre content in other tubers were as follows: Blesbok (5.47%), Bophelo (5.69%), Ndou (5.61%), Monate (5.60%) and 199062.1 (5.95%). The total fibre content in Impilo was significantly higher when compared with Blesbok, Bophelo, Ndou, Monate, Mvuvhelo and 199062.1 at $P<0.05$.

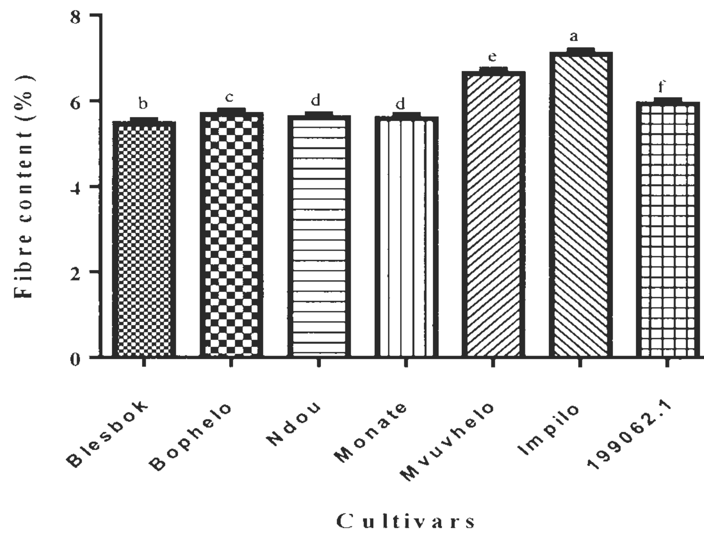


Figure 3.2: Fibre contents of tubers of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). Same letters indicate non-significant difference while different letters indicate significant difference at p<0.05.

3.3.3 Total ash content

The result (figure 3.3) shows that 199062.1 tubers had the highest ash content (5.81 %) followed by Blesbok (5.38 %). The levels of ash content in other tubers were as follows: Bophelo (5.03 %), Ndou (4.88 %), Monate (4.50 %), Mvuvhelo (4.95 %) and Impilo (4.87 %). The total ash content in 199062.1 was significantly higher when compared with Blesbok, Bophelo, Ndou, Monate, Mvuvhelo and Impilo at $p<0.05$.

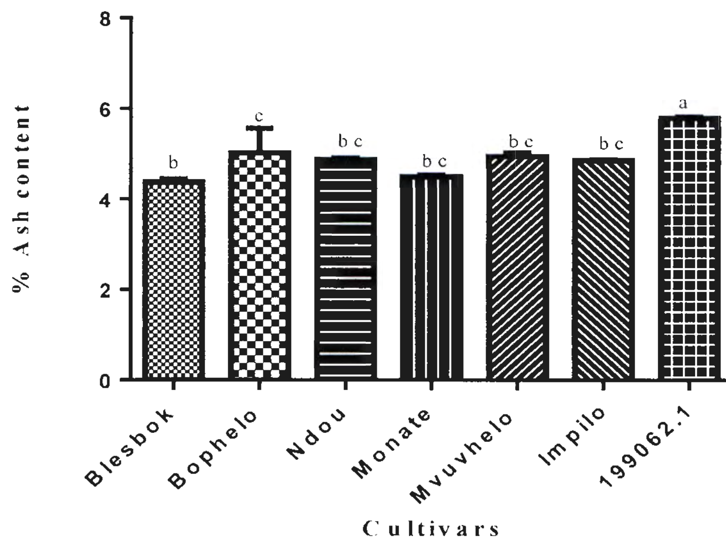


Figure 3.3: Ash contents of tubers of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). Same letters indicate non-significant difference while different letters indicate significant difference at $p<0.05$.

3.3.4 Total fatty acids composition

The results (Table 3.2) show that Bophelo had the highest total fatty acid content (0.650 %) with saturated fatty acid (0.270 %) mono-saturated fatty acid (0.050 %) and poly-saturated fatty acid (0.270 %). The lowest total fatty acid content was in Impilo (0.380 %) with saturated fatty acid (0.225 %), mono-saturated fatty acid (0.020 %) and poly-saturated fatty acid (0.135 %). Analysis of the fatty acid composition indicated the presence of five fatty acids (palmitic acid (C16), stearic acid (C18), arachidic acid (C20), oleic acid (C18:1), and linolenic acid (C18:2) in the tubers of all the cultivars of sweet potato in this study. It could be seen that the concentrations of C16 and C18:2 were more than C18, C20 and C18:2 in all the varieties of sweet potato. However, Monate had the highest C16 (0.257) followed by Bophelo (0.246 %) and then Blesbok (0.207 %) while the lowest concentration of C16 was in Impilo (0.157%). Bophelo had the highest C18:2 (0.282 %) followed by Blesbok (0.272 %) and then Monate (0.238 %) while the lowest C18:2 was in Impilo (0.120 %). The results also showed that the levels of total fatty acid and polyunsaturated fatty acid in Bophelo were significantly ($p<0.05$) higher than Ndou, Mvuvhelo, Impilo and 199062.1 but were not significantly different from Blesbok and Monate. The levels of saturated fatty acid in all the cultivars were not significantly different across all the cultivars. However, there was a significant increase ($p<0.05$) in the level of monounsaturated fatty acid in Blesbok when compared with other cultivars. The level of C16 in Monate was significantly higher ($p<0.05$) when compared with Ndou, Mvuvhelo, Impilo and 199062.1 and not significantly different with Blesbok and Bophelo while the level of C18 in Monate was significantly higher when compared with Bophelo, Ndou, Mvuvhelo, Impilo and 199062.1 and not significantly different with Blesbok. The results also showed no significant difference in the level of C20 in Bophelo in comparison to Ndou, Monate and Mvuvhelo while there was significant decrease ($p<0.05$) in Blesbok, Impilo and 199062.1. There was a significant increase ($p<0.05$) in the level of C18:1 in Blesbok when compared with other

cultivars. The levels of C18:2 in Bophelo were significantly higher ($p<0.05$) than Ndou, Monate, Mvuvhelo, Impilo and 199062.1 and were not significantly different from Monate.

Table 3.2: Fat contents (%) of the tubers of seven South African grown cultivars of sweet potato.

Cultivars	Saturated Fatty acids			Total Saturated fatty acid	Mono-unsaturated Fatty acids	Poly-unsaturated Fatty acids	Total Fatty acid (%)
	C16	C18	C20		C18:1	C18:2	
Blesbok	0.21± 0.01^{ab}	0.04± 0.00^{b}	0.04± 0.04^{b}	0.30± 0.01^{a}	0.05± 0.00^{a}	0.27± 0.01^{a}	0.60 ± 0.01^{a}
Bophelo	0.24± 0.00^{a}	0.04± 0.00^{b}	0.08± 0.00^{a}	0.34± 0.01^{a}	0.04 ± 0.00^{a}	0.28± 0.00^{a}	0.65±0.01^{a}
Ndou	0.19± 0.02^{ab}	0.03± 0.00^{b}	0.06± 0.00^{a}	0.28± 0.03^{a}	0.02 ± 0.00^{b}	0.17± 0.00^{b}	0.45 ± 0.02^{b}
Monate	0.26± 0.00^{a}	0.07± 0.00^{a}	0.05± 0.00^{b}	0.33± 0.07^{a}	0.02± 0.00^{b}	0.24± 0.01^{c}	0.62± 0.01^{a}
Mvuvhelo	0.21± 0.00^{ab}	0.03± 0.00^{b}	0.07± 0.00^{a}	0.26± 0.05^{a}	0.02± 0.00^{b}	0.18± 0.00^{b}	0.48± 0.00^{b}
Impilo	0.16± 0.02^{b}	0.02± 0.00^{b}	0.04± 0.00^{b}	0.23± 0.01^{a}	0.02± 0.00^{b}	0.13± 0.02^{d}	0.38± 0.01^{c}
199062.1	0.20± 0.01^{ab}	0.03± 0.00^{b}	0.05± 0.00^{b}	0.26± 0.02^{a}	0.02± 0.00^{b}	0.16± 0.01^{e}	0.43± 0.03^{b}

Values are mean ± SD (n=3). Same letters within each column indicate non-significant difference while different letters indicate significant difference at $p<0.05$

3.3.5 Total sugar, carbohydrate and energy values

Some starch in the sweet potato tubers are converted into reducing sugars and subsequently into sucrose during storage and hence, sucrose is the most abundant sugar in raw sweet potatoes with smaller amounts of glucose and fructose (Bouwkamp, 1985; Salunke and Kadam, 1998; Rose and Vasanthakaalam, 2011). In this study, the results (Table 3.3) show that Impilo had the highest total sugar content (22.21 %), containing fructose (3.99 %) glucose (3.71 %), sucrose (13.26 %) and maltose (1.25 %), followed by Blesbok with total sugar content (21.94 %) which had fructose (4.03 %) glucose (3.80 %), sucrose (12.84 %) and maltose (1.28 %). The sweet potato tuber with the lowest sugar content was Ndou; total sugar content (15.84%), fructose (1.75 %), glucose (0.92 %), sucrose (11.86 %) and maltose (1.31 %). The level of total sugar in Impilo was significantly higher than Bophelo, Ndou, Monate, Mvuvhelo and 199062.1 and not significantly different from Blesbok. The level of fructose in Blesbok was significantly higher ($p<0.05$) when compared with Bophelo, Ndou, Mvuvhelo and 199062.1 and not significantly different with Monate and Impilo while the level of glucose in Blesbok was significantly higher ($p<0.05$) when compared with Bophelo, Ndou, Monate, Mvuvhelo and 19906.1 and not significantly different with Impilo. The results also showed significant decrease ($P<0.05$) in the level of sucrose in 199062.1 in comparison with other cultivars while the level of maltose in Bophelo was not significantly different from other cultivars.

Furthermore, Figure 3.4 shows that Blesbok had the highest total carbohydrate content (76.72 %), followed by Ndou (76.09 %). The levels of total carbohydrate content in other tubers were Bophelo (69.42 %), Monate (75.98 %), Mvuvhelo (74. 51 %), Impilo (75.49 %) and 199062.1 (68.65 %). The total carbohydrate content in Blesbok was significantly higher ($p<0.05$) than Bophelo, Mvuvhelo, Impilo and 199062.1 and, was not significantly different in comparison with Ndou and Monate. The energy values (Figure 3.5) for all the sweet potato tubers were Blesbok (1446.42 KJ/100g), Bophelo (1458.42 KJ/100g), Ndou (1451.58 KJ/100g), Monate

(1564.15 KJ/100g), Mvuvhelo (1444.38 KJ/100g), Impilo (1444.51.47 KJ/100g) and 199062.1 (1421.69 KJ/100g). The energy contents of Bophelo and Ndou were significantly higher ($p<0.05$) than 199062.1 and not significantly different when compared with Blesbok, Monate, Mvuvhelo and Impilo.

Table 3.3: Sugar contents (%) of the tubers of seven South African grown cultivars of sweet potato.

Cultivars	Total Sugar (%)	Fructose (%)	Glucose(%)	Sucrose(%)	Maltose(%)
Blesbok	21.94± 0.56[a]	4.03± 0.07[a]	3.8± 0.81[a]	12.84± 0.10[bc]	1.28± 0.20[a]
Bophelo	17.54± 0.12[bc]	1.43± 0.02[b]	0.99± 0.08[b]	13.95± 0.05[b]	1.18± 0.07[a]
Ndou	15.84± 0.33[c]	1.75± 0.21[b]	0.92± 0.05[b]	11.86± 0.00[c]	1.31± 0.07[a]
Monate	20.5± 0.21[a]	3.77± 0.23[a]	2.70 ± 0.13[c]	12.97± 0.04[bc]	1.07± 0.11[a]
Mvuvhelo	16.74± 0.23[c]	1.45± 0.18[b]	0.92± 0.10[b]	13.1± 0.12[b]	1.26± 0.05[a]
Impilo	22.21± 0.23a	3.99± 0.10[a]	3.71± 0.21[a]	13.26± 0.02[b]	1.25± 0.09[a]
199062.1	19.02± 0.40[b]	1.52± 0.11[b]	1.46± 0.32[d]	14.8± 0.14[a]	1.24± 0.06[a]

Values are mean ± SD (n=3). Same letters within each column indicate non-significant difference while different letters indicate significant difference at $p<0.05$.

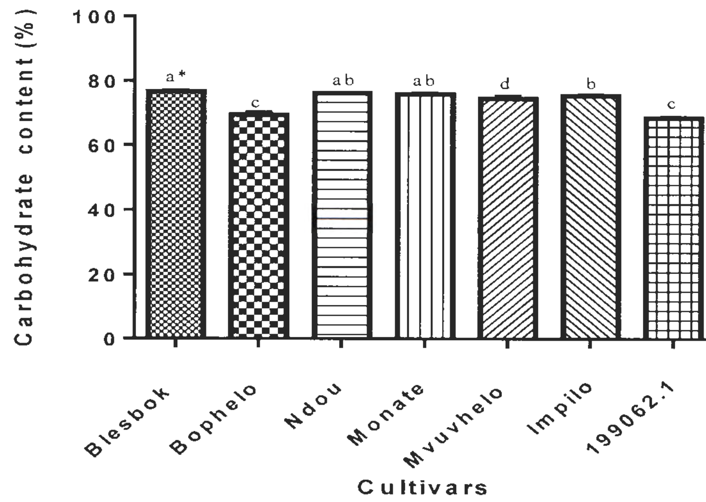


Figure 3.4: Carbohydrates content of the tubers of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). Same letters indicate non-significant difference while different letters indicate significant difference at $p<0.05$.

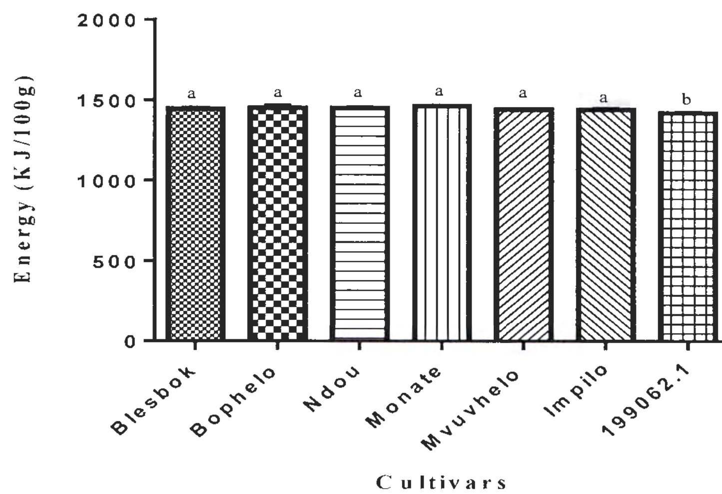


Figure 3.5: Energy contents of the tubers of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). Same letters indicate non-significant difference while different letters indicate significant difference at $p<0.05$

3.3.6 Metabolites detection with GC-MS

Different chemical constituents found in aqueous and aqueous-methanolic tuber extracts of the sweet potato used in this study are presented in Table 3.4. The chemical constituents are silane, trimethylpropoxy; silane, (2-furanylmethoxy) trimethyl; propanoic acid, 2-[(trimethylsilyl)oxy]; propanoic acid, 2-[(trimethylsilyl)oxy]- trimethylsilyl ester; glycolic-acid; cyclohexene, 3,3-dimethyl-1-(trimethylsilyloxy); pentasiloxane, dodecamethyl; 3,7-dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl; 4-Hydroxybutyric-Acid; silanol, trimethyl-,phosphate; furan-2-carboxylic acid, 3-methyl-,trimethylsilyl ester; glycerol, tris(trimethylsilyl) ether; butanedioic acid, bis(trimethylsilyl) ester; propanoic acid, 2,3- bis[(trimethylsilyl)oxy]-,trimethylsilyl ester; propanoic acid, 3-(trimethylsilyl)-, ethyl ester; 2-ketohexanoic acid, trimethylsilyl ester; 2-furanacetic acid, à-(trimethylsilyl)oxy]-, trimethylsilyl ester; a-eudesmol, trimethylsilyl ether; phosphonic acid, ethyl-, bis(trimethylsilyl) ester; malic acid; 2-aminophenol, O- tert.butyldimethylsilyl; gulonic acid, 2,3,5,6-tetrakis-O, (trimethylsilyl)-, lactone; myo-inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl); cinnamic acid, 3,4-bis(trimethylsiloxy); glycerol, tris(trimethylsilyl) ether; benzoic acid, 3,4,5-tris(trimethylsiloxy)-,trimethylsilyl ester; butanoic acid, 3-methyl-2-oxo-, trimethylsilyl ester.

Table 3.4a: Metabolites detected in the tubers of seven South African grown cultivars of sweet potato.

	CHEMICAL CONSTITUENTS	MASS	BLE		BOP		NDO		MON		MUV		IMP		199	
			A	AM	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM
1	Silane, trimethylpropoxy-	117	+	+	+	+	+	+	-	+	+	+	+	-	-	+
2	Silane, (2-furanylmethoxy)trimethyl-	81	+	+	+	+	+	+	-	+	+	+	+	-	-	+
3	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	117	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Glycolic-acid	147	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Cyclohexene, 3,3-dimethyl-1-(trimethylsilyloxy)-	183	+	+	+	+	+	+	-	+	+	+	+	-	-	+
6	Pentasiloxane, dodecamethyl-	281	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl-	103	+	+	+	+	+	+	-	+	+	+	+	-	-	+
8	4-Hydroxybutyric-Acid	147	+	-	-	-	-	+	-	+	+	+	+	+	+	-
9	Silanol, trimethyl-, phosphate	299	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3.4b: Metabolites detected in the tubers of seven South African grown cultivars of sweet potato.

	CHEMICAL CONSTITUENTS	MASS	BLE		BOP		NDO		MON		MUV		IMP		199	
			A	AM	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM
10	Furan-2-carboxylic acid, 3-methyl-,trimethylsilyl ester	109	+	+	-	+	+	+	-	+	+	+	+	-	-	+
11	Glycerol, tris(trimethylsilyl) ether	205	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Butanedioic acid, bis(trimethylsilyl) ester	55	+	+	+	+	+	+	+	-	+	-	+	+	+	-
13	Propanoic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	189	+	-	+	-	-	-	+	-	-	-	+	-	+	-
14	Propanoic acid, 3-(trimethylsilyl)-, ethyl ester	159	+	+	+	+	-	+	-	+	+	+	-	-	-	-
15	2-Ketohexanoic acid, trimethylsilyl ester	143	+	+	-	-	-	-	-	+	+	-	-	-	-	+

Table 3.4c: Metabolites detected in the tubers of seven South African grown cultivars of sweet potato.

	CHEMICAL CONSTITUENTS	MASS	BLE		BOP		NDO		MON		MUV		IMP		199	
			A	AM	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM
16	á-Eudesmol, trimethylsilyl ether	130	+	-	+	+	-	+	-	-	+	-	-	-	-	-
17	Phosphonic acid, ethyl-, bis(trimethylsilyl) ester	239	+	+	+	-	+	-	-	-	+	-	-	-	-	-
18	Malic-Acid	233	-	-	-	-	-	+	+	+	-	+	-	+	+	-
19	2-Aminophenol, O-tert.butyldimethylsilyl-	166	+	-	+	+	+	-	+	-	-	-	+	+	-	-
20	Gulonic acid, 2,3,5,6-tetrakis-O-(trimethylsilyl)-, lactone	81	-	-	-	-	-	-	+	-	-	-	-	-	-	-
21	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	217	+	+	+	+	+	-	+	-	+	-	+	+	+	+
22	Cinnamic acid, 3,4-bis(trimethylsiloxy)-, methyl ester	219	-	-	-	-	-	-	+	+	-	+	+	-	+	-
23	Glycerol, tris(trimethylsilyl) ether	205	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3.4d: Metabolites detected in the tubers of seven South African grown cultivars of sweet potato.

	CHEMICAL CONSTITUENTS	MASS	BLE		BOP		NDO		MON		MUV		IMP		199	
			A	AM	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM
24	Benzoic acid, 3,4,5-tris(trimethylsiloxy)-,trimethylsilyl ester	281	+	-	+	-	+	-	+	-	+	-	-	-	+	-
25	Butanoic acid, 3-methyl-2-oxo-, trimethylsilyl ester	214	-	-	-	-	-	-	-	-	-	-	-	+	-	-
26	D-Arabinonic acid, 2,3,5-tris-O-(trimethylsilyl)-, c-lactone	129	-	-	-	-	-	+	-	-	-	-	-	-	-	-
27	2-Furanacetic acid, à-[(trimethylsilyl)oxy]-, trimethylsilyl ester	169	+	+	+	-	-	-	-	-	+	-	+	-	-	+

BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1; A , Aqueous extracts; AM, Aqueous-methanol extracts; +, detected, ;- ,not detected

3.4 DISCUSSION

Proteins are needed in the body for repair and maintenance of body tissues, production of some hormones and as a source of energy generation. In this study, the protein contents of sweet potato cultivars used were higher than those of other cultivars from different geographical locations, ranging between 3.28 to 4.16% in Nigeria (Omodamiro *et al.* 2009), Bangladesh with values between 1.91-5.83% (Alam *et al.,* 2016), Brazil with values that ranged between 4.80 to 5.82% (Rodriques *et al.,* 2016), Rwanda with values ranging from 0.71 to 0.91% (Ingabire and Hilda, 2011) and 2.03 to 4.19% in Benin (Sanoussi *et al.,* 2016). The high levels of protein in these cultivars could be helpful in the reduction of kwashiorkor, a common protein-deficiency symptom among rural dwellers.

Similarly, the values obtained for fiber content in this study are higher than those of Omodamiro *et al.* (2013) in Nigeria which ranged between 0.67 - 2.00%, Alam *et al.* (2016) in Bangladesh between 0.30 - 0.54%, Rodriguez *et al.* (2016) in Brazil 2.57%, Sanoussi *et al.* (2016) in Benin between 2.56 - 4.70% and Ingabire and Hilda, (2011) in Rwanda between 0.11 - 0.14%. High dietary fibre content is vital for digestibility enhancement, reduced blood cholesterol and decline of the risk of large bowel cancers (Al-Farga *et al.,* 2016). It has also been suggested that fibre can exert a wide range of benefits in areas such as bowel function, gut health, immunity, blood glucose control, and serum lipid levels (Okaka *et al*., 2006; Agbafor *et al*., 2015).

The values obtained for fatty acid content in this study were lower than those of Omodamiro *et al.* (2013) and Anthony *et al.* (2014) in Nigeria ranging from 1.02 to 1.72% and 1.41 to 2.92 respectively. However, they were comparable with 0.39% value in Brazil (Rodriguez *et al.,* 2016) and higher than 0.11 - 0.14% in Bangladesh (Alam, 2016). Reduced fat content in food is desirable as they are beneficial diet in certain metabolic diseases such as diabetes mellitus, cardiovascular diseases and obesity. In this study, different fatty acids were found in the cultivars such as palmitic

acid (C16, a saturated long chain fatty acid with sixteen carbon backbone), stearic acid (C18, a saturated fatty acid having 18-carbon chain), arachidic acid (C20, a saturated long-chain fatty acid with 20-carbon backbone), oleic acid (C18:1, a monounsaturated omega-9- fatty acid having 18-carbon chain with a single bond), and linoleic acid (C18:2, a polyunsaturated omega-6 fatty acid having 18-carbon chain with two double bonds). Stearic acid has a neutral effect on total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol unlike palmitic acid that has been reported to be hypercholesterolemic (Godswill *et al.*, 2016). However, palmitic acid is only destructive if the concentration of linoleic acid in the diet is quite low (Godswill *et al.*, 2016). Oleic acid has preventive effect on ulcerative colitis, protects the cells from free radical damage as well as decreasing blood pressure and increasing fat burning (Adegbe *et al.*, 2016).

The ash content is an indication of the presence of some minerals in the cultivars. Minerals are very essential and synergistically work with vitamins, enzymes, hormones and other nutrient cofactors to effectively regulate many biological functions in the body (Hamza *et al.*, 2014). The values obtained for ash content in this study are higher than those of Omodamiro *et al.* (2013) and Anthony *et al,* (2014) in Nigeria ranging from 0.50 – 1.52% and 2.27 – 3.10% respectively as well as Alam *et al.* (2016) in Bangladesh between 1.17 – 1.31%, Rodriguez *et al.* (2016) in Brazil between 2.04 – 3.80% and Sanoussi *et al.* (2016) in Benin between 2.56 - 4.70%. This indicates that the cultivars are good source of minerals which can be helpful in promoting health. Similarly, the values obtained for carbohydrate content in the cultivars used in this study were higher than those from Nigeria which ranged between 20.28 - 35.12% (Omodamiro *et al.*, 2013) and 21.10 - 24.50% in Bangladesh (Alam *et al.*, 2016). They were also lower than those from Brazil between 85.80 - 90.17%, and 90.24 - 176.96% in Benin (Sanoussi *et al.*, 2016). Carbohydrates, which can be converted to simple forms of

sugar such as glucose, fructose and galactose (with fructose and galactose finally converted to glucose), play an important role in the supply of energy for metabolic processes in the body. The sugar content and the energy values of the sweet potato cultivars in this are comparable with literature reports.

Some reported health benefits of the chemical constituents found in the sweet potato cultivars include glycolic acid, used in cosmetics and dermatology (Babilas *et al*., 2012), propanoic acid, which has antioxidant and antiproliferative effects (Suprayitno, 2015, Kifayatullah *et al*., 2016), benzoic acid, having antibacterial, antioxidant, anticancer, antiseptic; antiviral and hepatoprotective potentials (Elmezian *et al*., 2016) and malic acid which has been therapeutically used in combination with benzoic acid and salicylic acid to treat bums, ulcers, wounds as well as in the treatment of liver disorders and as a drug that promotes the secretion of saliva (Brittain, 2010). In addition, cinnamic acids have been shown to be a promising antioxidant (Pontiki *et al*., 2014) and anticancer compounds (Niero *et al*., 2013, Pontiki *et al*., 2014).

3.5 CONCLUSION

Based on the findings of this study, it can be concluded that the South Africa grown cultivars of sweet potato analysed have good nutritional components such as crude protein, ash, fibre and carbohydrates that make them very promising alternative source of food to overcome malnutrition. The orange-flesh sweet potatoes cultivars (Bophelo, Impilo, 199062.1) showed the highest nutritional potentials for most of the parameters measured. However, the white fleshed cultivars (Blesbok, Ndou and Monate) generally contain more total carbohydrates than the orange fleshed cultivars. In addition, the beneficial phytochemicals detected in some of the cultivars supports the therapeutic reports of sweet potato. Access to these compounds in daily diets can help to fight against various diseases and maintain health and wellness.

CHAPTER FOUR

COMPARATIVE ANALYSIS OF THE ANTIOXIDANT PROFILES AND ACTIVITY OF SELECTED SOUTH AFRICAN GROWN CULTIVARS OF SWEET POTATO

ABSTRACT

The antioxidant profile and activity of aqueous and aqueous methanol extracts of four white fleshed (Blesbok, Ndou, Monate and Muvhelo) and three orange fleshed (Bophelo, Impilo and 199062.1) sweet potato cultivars grown in South Africa were investigated. The total flavonol, total flavanol, total flavonoid and total polyphenol content of the extracts were determined spectrophotometically using established techniques. The antioxidant activities of the extracts were determined using the ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) and nitric oxide (NO) scavenging assay methods. Specific polyphenols (caffeic acid, catechin, hyperoside, iso-orientin, kaempferol, quercetin, rutin, orientin, vitexin, protocatechuic acid, vanyllic acid, isovanillic acid) and ascorbic acid were also quantified using liquid chromatography (LC) coupled with mass spectrometry (MS). There were significant variations in the antioxidant profiles and activities across the cultivars. In the tubers, the aqueous extracts of Bophelo had the highest profile except the total flavonoid with highest amount in the aqueous extract of 199062.1. In the leaves, the aqueous-methanol extract of Bophelo also had the highest antioxidant profile with the exception of total flavanol content which was found to be the highest in aqueous extract of Blesbok. Similarly, the aqueous and aqueous-methanol extracts of Bophelo consistently exhibited the strongest antioxidant activity in the tubers and leaves respectively as determined by the TEAC and FRAP methods. The aqueous extract of 199062.1 and aqueous-methanol extract of Bophelo exhibited the highest radical scavenging activity towards nitric oxide in the tubers and leaves respectively. Generally, the orange fleshed cultivars are richer in antioxidants than their white fleshed counterparts, while the leaves extracts contained higher

concentration of antioxidant compounds and stronger antioxidant activities than the tubers extracts. Of all the specific phenolic compounds quantified, hyperoside and caffeic acid were the most abundant compounds found in the leaves and tuber extracts respectively. The highest amounts of these compounds were found in the aqueous methanol extracts of Bophelo. Generally, the compounds were more accumulated in the leaves than in the tubers. The least detected compounds were kaempferol, catechin, vitexin and orientin. Orange fleshed sweet potatoes, like Bophelo, can be explored for their relatively high antioxidant profile and activity in oxidative stress mediated diseases.

4.1 INTRODUCTION

Current interest in controlling and preventing degenerative diseases through nutraceuticals has promoted research on the bioactive components of edible plants. Several plants, including vegetables and fruits, have been confirmed to contain antioxidants which can be beneficial to human health. Oxidative stress has been implicated in different metabolic disease process including diabetes (Le lay *et al.*, 2014). Hence, strategies to prevent and ameliorate oxidative stress remain important in the overall treatment of these diseases (Henriksen *et al.*, 2011). Beyond their traditional nutritional value, food crops can as well be exploited for their antioxidants contents and medicinal potential. Sweet potato is a global food crop with phytochemicals that are beneficial to human health (Islam, 2014). The bioactivity of sweet potato has been attributed to the beneficial phytochemicals present in the extracts. Phytochemicals in sweet potato include phenolic acids, flavanoids, and ascorbic acids (Grace *et al.*, 2014). Flavonoids are the most abundant polyphenols in human diets, accounting for more than half of the eight thousand natural phenolic compounds found in common fruits and vegetables (Ozcan *et al.*, 2014). They include flavonols, flavanones, flavones and flavanols and make up the majority of plant secondary metabolites (Magiera and Zareba, 2015). Flavonoids are notable for their antioxidant property which makes them good therapeutic agents (Sangeetha *et al.*, 2016; Kumar and Pandey, 2013). Specific flavonoids such as rutin, kaempeferol, quercetin and orientin have been reported to exhibit biological activities such as antitumour, antimicrobial, antidiabetes and antiinflammatory (Kim *et al.*, 2004; Kumar and Pandey *et al.*, 2013; Dhanya *et al.*, 2014). Common flavonoids that have been found in sweet potato are quercetin, myricetin, luteolin, apigenin, and kaempferol (Ojong *et al.*, 2008). Phenolic acids are widely found in plants including fruits and vegetables and they have been associated with nutritional and antioxidant properties of foods (Robbins, 2003). Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acid, they may also occur in edible plants as

glycosides or esters with other natural compounds such as alcohols, sterols and hydroxyfatty acids (Ghasemzadeh and Ghasemzadeh, 2011). Phenolic acids that have been detected in sweet potato roots and leaves include caffeoylquinic acid, dicaffeoylquinic acid, p-coumaroylquinic, and feruloylquinic acid (Nandutu *et al.*, 2007, Padda and Picha, 2007). Ascorbic acid is an antioxidant molecule which performs important metabolic functions in animals, single cell organisms and plants (Mazid *et al.*, 2011). It is a strong reducing agent that can effectively scavenge harmful free radicals generated in the body (Padayatty *et al.*, 2003). Ascorbic acid has the potential to act against inflammation, oxidative stress and the resulting oxidative damage that can lead to the development and progression of numerous metabolic diseases (Grosso *et al.*, 2013). The quantity of phenolic compounds and other important phytochemicals in sweet potato, as in any other plant, varies from one cultivar to the other (Padda and Picha, 2008; Shekar *et al.*, 2015). Proper investigation of these variations can lead to identification of cultivars with high level of phenolic compounds which can be useful in breeding programs for improved profile of useful phenolic compounds in sweet potato. This study investigated the antioxidant profile and activity of the extracts of the leaves and tubers of seven South African grown cultivars of sweet potato. Furthermore, it determined the quantity and variation of specific flavonoids, phenolic acids and ascorbic acid in the extracts relative to the standard compounds.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

Sweet potato cultivars (Table 4.1) were obtained from Agricultural Research Council (ARC), South Africa, through the Department of Crop Science, North West University, Mafikeng campus, South Africa. The leaves and root tubers of each cultivar were collected fresh from the university farm. They were rinsed under running water. The leaves were cut into pieces and tubers into thin chips before being air dried in the laboratory at room temperature ($25 \pm 2^{o}C$). Air dried samples were ground into fine

powder (about 500μm mesh size) and packed in an air tight container. They were labeled accordingly and stored at 4°C for further use.

Table 4.1: List of selected South African grown sweet potato cultivars

Cultivars	Skin colour	Flesh colour
Blesbok	Purple	White
Bophelo	Orange	Dark orange
Ndou	Cream	Cream
Monate	Cream	Cream
Mvuvhelo	Cream	Cream
Impilo	Deep orange	Light orange
199062-1	Cream	Light orange

4.2.2 Preparation of extracts

Preparation of crude aqueous and aqueous/methanolic (1:1) extracts of the leaves and tubers of all the cultivars were done by soaking 5g of the powder in 100ml of the respective solvents for 24 hours at room temperature with constant shaking. Extracts were recovered through lyophilization and evaporation using a freeze dryer (Alpha 1-4 LSC Plus) and a rotary evaporator (RE-52A) respectively. Dried extracts were stored in the dark at -20°C. Determination of the antioxidant content was done using extracts at 1mg/ml (leaves) and 10mg/ml (tubers), while concentrations of 0.5mg/ml (leaves) and 10mg/ml (tubers) were used for the antioxidant activity.

4.2.3 Antioxidant profiles

4.2.3.1 Determination of flavonol content

The flavonol content was determined according to the method of Mazza *et al.* (1999) with slight modifications. A sample volume (12.5μl) of each extract was added to 12.5μl of 0.1% HCl in 95% ethanol and 225μl of 2% HCl in designated wells of a 96 well plate. Absorbances of the resulting solutions were read at 360nm with Multiskan^{TM} spectrophotometer (Thermo scientific) after incubation for 30 minutes at room temperature. Quantification was based on standard curve generated from 0, 5, 10, 20, 40, 80 mg/L solutions of quercetin in 95% ethanol. Total flavonol content was calculated and expressed as mg of quercetin equivalent /g of extract.

4.2.3.2 Determination of flavanol content

A modified method of Treutter (1989) was adopted to estimate the total flavanol content of the samples using 4-(Dimethylamino)-cinnamaldehyde (DMACA) reagent. 0.05% DMACA solution was prepared in HCl-MeOH mixture (1:3). A sample volume of 50 μl of each exract was added to 250μl of DMACA solution in the wells, the plate was incubated for 30 minutes at room temperature and absorbances were

read at 640nm using Multiskan™ spectrophotometer (Thermo scientific). A standard curve was prepared using 0, 1.36, 2.72, 6.8, 13.6, 27.2mg/L solutions of catechin in methanol. Total flavanol contents of the samples were extrapolated from the standard curve and expressed as mg catechin equivalent /g of extract.

4.2.3.3 Determination of total flavonoid content

The total flavonoid content was estimated spectrophotometrically by adding 500µl of 2% aluminium chloride ($AlCl_3$) in ethanol to 500µl of the samples. The mixtures were incubated for 60 minutes at room temperature and the absorbances were measured at 420nm. Quantification of the total flavonoid contents of the samples was based on standard curve generated from 0, 10, 20, 40, 80, 160 mg/L solutions of quercetin in 95% ethanol. Results were expressed as mg of quercetin equivalent /g of extract.

4.2.3.4 Determination of total polyphenols content

The total polyphenol contents of the cultivars were determined spectrophotometrically using Folin-Ciocalteu method (Swain and Hillis, 1959) with slight modification. A sample volume of 25µl of the reconstituted extracts of the leaves and the tubers were added to 125µl of 10% folin reagent in a 96 well plate. After 5 minutes, 100µl of 7.5% Na_2CO3 was added to the wells and incubated for 2 hours at room temperature. The absorbances of the resulting solution were read at 765nm. Standard curve was generated using concentrations; 0, 20, 50, 100, 250, 500mg/l of gallic acid in 10% ethanol. The total polyphenols content were expressed as mg of gallic acid equivalent (GAE) /g of extract.

4.2.4 Antioxidant activity

4.2.4.1 Ferric Reducing antioxidant power (FRAP) assay

Frap assay was carried out according to the method of Benzie and Strain (1996). FRAP reagent was prepared by mixing 30ml acetate buffer, 3ml TPTZ solution, 3ml $FeCl_3$ solution and 6.6ml distilled water. Ten microliters (10µl) of sample and 300µl of FRAP reagent were added to the well and incubated at 37°C for 30 minutes. Absorbances were read at 593nm using Multiskan™ spectrophotometer (Thermo scientific). Aqueous solutions of 0, 50, 100, 200, 500, 1000µM ascorbic acid were used to prepare the standard curve. Results were expressed as µmol AAE/g of extract.

4.2.4.2 Trolox equivalent Antioxidant capacity (TEAC) assay

The trolox equivalent antioxidant capacity of the extracts was determined spectrophotometrically by the method of Re *et al.* (1999). ABTS radical cations were produced by reacting 88µl of the potassium-peroxodisulphate solution (7mM) and 5 ml of the ABTS solution (140mM). The mixture was left in the dark at room temperature for 24 hours and then diluted with ethanol in ratio 1:20 before use. Three hundred microliters (300µl) of the diluted ABTS was added with 25µl of the samples and incubated for 30 minutes at room temperature, absorbance was read at 734nm. A standard curve was prepared by using 0, 50, 100, 150, 250, 500µM solution of Trolox in ethanol. TEAC values were extrapolated from the standard curve and expressed as µmol TE/g of extract.

4.2.4.3 Nitric oxide (NO) Radical scavenging assay

The nitric oxide radical scavenging activity was measured using different concentrations of the extracts (Leaves- 0.125-1mg/ml and Tubers-1.25-10mg/ml), 5mM Nitropruside in phosphate buffer saline (pH 7.4) and Griess reagent. Griess reagent was prepared by mixing 1% sulphanilamide in 5% H_3PO_4 and

0.1% N-1-naphthylethylenediamine dihydrochloride (NED). The assay was done by adding 50µl of Nitropruside and 100µl of Griess reagent to 50µl of the extracts. The absorbances were measured at 532nm after 60 minutes of incubation. % inhibition was calculated as $(A_0-A_s)/ A_0 *100$ where A_0 is absorbance of control, A_s is the absorbance of samples. IC_{50} for each sample was estimated using linear regression analysis.

4.2.5 Quantitative Liquid Chromatography (LC) analyses

4.2.5.1 Preparation of samples and standard solutions

Stock solutions (100µg/ml) of the standards were prepared by dissolution in distilled water except for kaempeferol which was dissolved in a mixture of water, isopropanol and acetonitrile. Dried extracts were reconstituted to make 1mg/ml and 10mg/ml extracts of leaves and tubers respectively in the mobile phase solvent and filtered through a 0.45µm syringe filter system before injection into the LC system.

4.2.5.2 Chemicals

The phenolic acids (caffeic acid, isovanillic acid, vanillic acid and protocatechuic acid), flavonoids (rutin, hyperoside, orientin, iso-orientin, vitexin, quercetin, catechin and kaempferol) were purchased from Sigma-Aldrich, South Africa. Acetonitrile (BJ015CS) and water (BJ365CS) were purchased from Anatech Instruments (Pty) Ltd (Olivedale, South Africa). 2-acetamidophenol (A7000) and formic acid (14265) were purchased from Sigma-Aldrich (Kempton Park, South Africa).

4.2.5.3 Instrumentation and analysis

HPLC analysis was conducted on Agilent 1200 LC system (Agilent Technologies, Santa Clara, USA). Separation was achieved by injecting one microliter of sample into a Waters HSS T3 column (2.1 x 100 mm, 1.8 µm) (Microsep, Bramley, South Africa), at 30°C. The mobile phases were comprised of water

NWU LIBRARY

(solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid. The gradient used in separating the polyphenols is given in Table 4.2. Mass spectrometry detection was performed on an Agilent 6410 Triple Quadrupole (Santa Clara, USA) using positive electrospray ionisation. The drying gas temperature is used at 300°C with a drying gas flow of 7.5 L/min and nebuliser pressure of 30 psi. Mass spectrometry conditions were optimised with Agilent Technologies MassHunter optimizer software (B.04.01) using the standards. Detected peaks were identified and quantified by comparing the retention time and peak area to that of known standards.

Table 4.2: Mobile phase gradient elution program

Time (min)	Flow rate (ml/min)	Mobile Phase B (%)
0	0.2	5
1.5	0.2	5
6	0.2	20
16	0.2	100
17	0.3	100
18	0.2	5

4.2.6 Statistical analysis

All assays were carried out in triplicates and results are presented as mean ± standard deviation. Statistical analysis was done by GraphPad prism 5 statistical package (GraphPad Software, USA), and significant differences among the samples were calculated using one-way ANOVA followed by Tukeys test at $P < 0.05$. Pearson's correlation coefficient was calculated using Microsoft excel 2010.

4.3 RESULTS AND DISCUSSIONS

4.3.1 Antioxidant profiles

Polyphenols are beneficial phytochemicals which are characteristic contents of most medicinal plants (Pfundstein *et al.*, 2010). Flavonols, flavanals and flavonoids are all groups of polypenolic compounds found in plants. Generally, polyphenolic compounds are regarded as potent antioxidants, therefore their presence and concentrations in a plant is an important index of the antioxidant ability of the plant (Maksimovic *et al.*, 2005). This study investigated the total flavonols, total flavanols, total flavonoids and total polyphenol content of the tubers' and leaves' extracts of seven sweet potato cultivars grown in the same geographical area and under the same condition in order to determine their antioxidant profiles. Aqueous and aqueous- methanol extracts of the samples were used in the study. Antioxidant profiles were determined spectrophotometrically with established techniques.

The results for the total flavonols, total flavanols, total flavonoids and total polyphenols of the tubers' extracts are shown in Table 4.3. Total flavonol content (TFVO) of the tubers' extracts ranges from 0.06 ± 0.05 mgQE/ per g extract in the aqueous-methanol extract of Blesbok to 1.01 ± 0.24 mgQE/per g extract in the aqueous extract of Bophelo. Aqueous extracts of Impilo and 199062.1 and the aqueous-methanol extract of Monate cultivars also have appreciable TFVO of 0.83 ± 0.18 mgQE/per g extract, 0.85 ± 0.08 mgQE/per g extract and 0.97 ± 0.14 mgQE/per g extract respectively which were not significantly different from that of Bophelo at $p>0.05$. The highest total flavanol content (TFVA) was also found in the aqueous extract of Bophelo with a value of 0.82 ± 0.01 mgQE/per g extract while the lowest value of 0.02 ± 0.00 mgQE/per g extract was found in both aqueous and aqueous methanol of Blesbok. Aqueous extract of Impilo also had a considerable TFVA of 0.79 ± 0.04 mgQE/per g extract which was not significantly ($p>0.05$) different from that of Bophelo. However, the aqueous-methanol extract had a significantly ($P<0.05$) lower TFVA of 0.03 ± 0.00 mgQE/per g extract. Total flavonoids

(TFV) content of the tubers' extract ranges from 0.15 ± 0.02 mgQE/g extract in aqueous-methanol of Ndou to 3.65 ± 0.06 mgQE/g extract in the aqueous extract of 199062.1. Aqueous extract of Bophelo and Impilo also had appreciable TFV values of 3.31 ± 0.13 mgQE/g extract and 2.43 ± 0.06 mgQE/g extract respectively. The highest total polyphenol (TPO) content was found in the aqueous extract of Bophelo with value of 10.96 ± 0.66 mg GAE/g extract. The aqueous- methanol extract of Bophelo also had considerable TPO value of 7.36 ± 0.66 mg GAE)/g extract which was not significantly different from that of the aqueous extract at ($p>0.05$). These values were significantly higher than those of other cultivars. The aqueous-methanol extract of Ndou (2.33 ± 0.05 mg GAE)/g extract) and the aqueous extract of Blesbok (2.42 ± 0.35 mg GAE)/g extract) had the lowest values of total polyphenol content.

Generally, the tubers of the orange fleshed cultivars had higher antioxidant profile than the white fleshed cultivars. This finding is consistent with that of Tang *et al.* (2015) who investigated the total polyphenol content of tubers of sweet potato cultivars with different flesh colours and reported a significantly higher value (6.17 ± 0.12mg GAE/g) in the orange fleshed cultivar than in the white fleshed cultivars (4.70 ± 0.10mg GAE/g). Among the orange-fleshed cultivars, Bophelo consistently contain higher content of polyphenols which suggests that even among cultivars of the same flesh colour, the polyphenol content can still differ sometimes even significantly (Ayeleso *et al.*, 2016).

Table 4.4 shows the results for the total flavonols, total flavanols, total flavonoids and total polyphenols of the leaves' extracts. In the leaves' extracts, TFVO range from 8.05 ± 0.39 mg QE/g extract in aqueous extract of Ndou to 52.53 ± 3.12 mg QE/g extract in aqueous methanol extract of Bophelo. TFVA in the leaves' extract range from 0.10 ± 0.01 mg CE/g extract in aqueous methanol extract of Ndou to 0.35 ± 0.02 mg CE/g extract in aqueous extract of Blesbok. Bophelo also had considerable TFVA of 0.33 ± 0.00 mg CE/g extract and 0.28 ± 0.01mg CE/g extract in the aqueous and aqueous-methanol extracts respectively. TFV content was found to be highest in the aqueous methanol extract of

Bophelo (85.28 ± 3.27mg QE/g extract) and lowest in aqueous-methanol extract of Ndou (33.33 ± 2.80mg QE/g extract). Similarly, the highest TPO content was also found in aqueous-methanol extract of Bophelo (152.20 ± 3.52 mg GAE/g extract) and the lowest in aqueous extract of Ndou (46.12 ± 1.11 mg GAE/g extract).

Bophelo and Ndou consistently showed significantly ($P<0.05$) higher and lower contents of the antioxidant profiles respectively than all the other cultivars except in the total flavanol contents. Furthermore, across all the parameters measured in the leaves' extracts, the aqueous-methanol extract mostly had higher antioxidant content than the aqueous extract in each of the cultivars. This suggests that a mixture of methanol and water (ratio 1:1) was able to extract the polyphenolic compounds in the leaves better than only water. This is possibly due to the fact that methanol is a less polar solvent than water, its mixture with water would allow the extraction of other less polar compounds, thus, resulting into higher concentration of antioxidant compounds than in aqueous extract. Solvent is an important factor which is very influential on the extraction efficiency of polyphenolic compounds (Rebaya *et al*., 2014; Fu *et al*., 2016). The study of Fu *et al*. (2016) indicated that extracting solvents significantly influence the solubility of phenolic compounds in sweet potato leaves, and thus play a key role in the extraction efficiency of these compounds. Babbar *et al*. (2014) also demonstrated the influence of the choice of solvent on total phenols in plant where they reported that methanolic extract of the peels of 4 vegetables showed higher polyphenolic content than other extracts (hexane, chloroform and ethyl acetate). Moreover, there is a clear indication that not only the tubers but also the leaves of the orange-fleshed sweet potato cultivars contain more polyphenols than those of the white fleshed cultivars used in this study. Another finding from this study which is consistent with that of Padda and Picha (2007) and Islam *et al*. (2002) is that the leaves of the sweet potato cultivars had significantly higher content of polyphenols and stronger antioxidant activity than the tubers. In this study, the highest total polyphenol

content in the leaves (152.20 ± 3.52 mg GAE/g extract) is more than 13 folds of the highest total polyphenol in the tubers (10.96 ± 0.66 mg GAE/g extract).

Table 4.3: Total flavonol, flavanol, flavonoid and polyphenol contents of the tubers of seven South African grown cultivars of sweet potato.

EXTRACTS	CULTIVARS	FLAVONOLS (mg QE/g extract)	FLAVANOLS (mg CE/g extract)	FLAVONOIDS (mg QE/g extract)	POLYPHENOLS (mg GAE/g extract)
AQ	BLE	0.33 ± 0.06^{ac}	0.02 ± 0.00^{a}	0.71 ± 0.02^{a}	2.42 ± 0.35^{a}
	BOP	1.01 ± 0.24^{b}	0.82 ± 0.01^{b}	3.31 ± 0.13^{b}	10.96 ± 0.66^{b}
	NDO	0.34 ± 0.12^{ac}	0.04 ± 0.00^{a}	0.58 ± 0.05^{a}	2.89 ± 0.29^{ac}
	MON	0.74 ± 0.15^{ab}	0.14 ± 0.01^{c}	1.44 ± 0.06^{c}	3.74 ± 0.57^{c}
	MUV	0.40 ± 0.07^{ac}	0.07 ± 0.00^{a}	0.55 ± 0.06^{a}	3.26 ± 0.33^{ac}
	IMP	0.83 ± 0.18^{b}	0.79 ± 0.04^{b}	2.43 ± 0.06^{d}	3.54 ± 0.13^{ac}
	199	0.85 ± 0.08^{b}	0.33 ± 0.02^{d}	3.65 ± 0.06^{e}	5.49 ± 0.19^{d}
AQ-ME	BLE	0.06 ± 0.05^{c}	0.02 ± 0.00^{a}	1.01 ± 0.02^{f}	2.50 ± 0.13^{a}
	BOP	0.44 ± 0.00^{ac}	0.10 ± 0.00^{c}	0.70 ± 0.07^{a}	7.36 ± 0.66^{e}
	NDO	0.57 ± 0.22^{ab}	0.05 ± 0.02^{a}	0.67 ± 0.05^{a}	2.33 ± 0.05^{a}
	MON	0.97 ± 0.14^{b}	0.06 ± 0.01^{a}	1.52 ± 0.03^{c}	2.76 ± 0.30^{ac}
	MUV	0.40 ± 0.04^{ac}	0.09 ± 0.00^{c}	1.18 ± 0.07^{f}	2.47 ± 0.30^{a}
	IMP	0.13 ± 0.03^{c}	0.03 ± 0.00^{a}	0.15 ± 0.02^{g}	3.72 ± 0.45^{c}
	199	0.59 ± 0.35^{ab}	0.08 ± 0.00^{c}	0.93 ± 0.05^{f}	2.90 ± 0.22^{ac}

Values are mean ± SD (n=3). Same letters and different letters across the groups (down the vertical column) indicate no significant and significant difference respectively at P<0.05. BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract.

Table 4.4: Total flavonol, flavanol, flavonoid and polyphenol contents of the leaves of seven South African grown cultivars of sweet potato.

EXTRACTS	CULTIVARS	FLAVONOLS (mg QE/g extract)	FLAVANOLS (mg CE/g extract)	FLAVONOIDS (mg QE/g extract)	POLYPHENOLS (mg GAE/g extract)
AQ	BLE	16.82 ± 1.36^{a}	0.35 ± 0.02^{a}	53.38 ± 1.97^{a}	69.27 ± 1.88^{ad}
	BOP	31.21 ± 2.71^{b}	0.33 ± 0.00^{a}	71.54 ± 4.33^{b}	82.04 ± 1.51^{b}
	NDO	8.05 ± 0.39^{c}	0.23 ± 0.02^{bc}	41.4 ± 0.858^{c}	46.12 ± 1.11^{c}
	MON	25.59 ± 2.57^{bd}	0.27 ± 0.02^{b}	63.97 ± 1.60^{d}	70.45 ± 3.56^{ad}
	MUV	12.52 ± 0.39^{ac}	0.21 ± 0.01^{c}	42.59 ± 1.40^{c}	63.33 ± 7.58^{a}
	IMP	25.92 ± 1.49^{d}	0.27 ± 0.01^{b}	67.38 ± 0.99^{bd}	74.86 ± 1.28^{d}
	199	20.14 ± 2.76^{d}	0.19 ± 0.03^{c}	54.66 ± 2.08^{a}	65.27 ± 1.33^{ad}
AQ-ME	BLE	31.18 ± 2.41^{b}	0.20 ± 0.01^{c}	62.50 ± 1.59^{d}	107.20 ±3.68^{e}
	BOP	52.53 ± 3.12^{e}	0.28 ± 0.01^{b}	85.28 ± 3.27^{e}	152.20 ± 3.52^{f}
	NDO	11.5 ± 2.43^{ac}	0.10 ± 0.01^{d}	33.33 ± 2.80^{f}	58.25 ± 1.61^{g}
	MON	44.14 ± 2.86^{e}	0.21 ± 0.03^{c}	77.84 ± 3.58^{b}	125.20 ± 2.47^{h}
	MUV	23.94 ± 2.85^{bd}	0.13 ± 0.01^{d}	53.91 ± 1.83^{a}	92.50 ± 3.24^{i}
	IMP	45.39 ± 2.32^{e}	0.23 ± 0.02^{bc}	81.49 ± 1.45^{e}	126.80 ± 3.87^{h}
	199	44.44 ± 4.80^{e}	0.30 ± 0.02^{ab}	76.03 ± 1.92^{b}	101.30 ± 6.46^{e}

Values are mean ± SD (n=3). Same letters and different letters across the groups (down the vertical column) indicate no significant and significant difference respectively at $P<0.05$. BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199,199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract.

4.3.2 Antioxidant activity

The results for the antioxidant activity as determined by the FRAP and TEAC methods are shown in Tables 4.5 and 4.6 for the tubers and leaves extracts respectively. FRAP assay measures the ability of the extracts to reduce the ferric iron complex to its ferrous form (Benzie and Strain, 1996). TEAC assay measures the ability of the extracts to scavenge the (ABTS) radical (ABTS•) thereby converting it into a colorless product (Arts *et al*., 2004). With both methods, Bophelo exhibited the strongest antioxidant activity which was significant at $p<0.05$ when compared to the other cultivars. In the tubers, the aqueous extract of Bophelo showed the highest antioxidant activities (222.60 ± 8.85 μmol AAE/g extract, 33.72 ± 1.70 μmol TE/g extract) as determined by FRAP and TEAC methods respectively. However, the lowest antioxidant activities were exhibited by the aqueous-methanol of Mvuvhelo (12.55 ± 0.18 μmol AAE/g) and aqueous extract of Blesbok (12.45 ± 4.49 μmol TE/g extract) by FRAP and TEAC methods respectively. In the leaves, the aqueous-methanol extract of Bophelo had the highest FRAP value of 844.10 ± 7.60 μmol AAE/g and also the highest TEAC value of 109.50 ± 4.91 μmol TE/g extract. On the other hand, the aqueous extract of Mvuvhelo had the lowest values of both FRAP (109.50 ± 4.91μmol AAE/g) and TEAC (278.30 ± 6.39μmolTE/g extract) antioxidant activities.

Nitric oxide is a ubiquitously distributed free radical in tissues and organs that have been implicated in pathological conditions (Brian *et al*., 2009). The nitric oxide scavenging (NO) assay was also carried out to determine the ability of the extracts to scavenge the NO radical. NO assay is also an index of the antioxidant capability of a compound and it is measured as percentage inhibition of NO radical. The percentage inhibition of the NO radical by the extracts at different concentrations (0.125-1) mg/ml and (1.25-10) mg/ml for leaves and tubers respectively are shown in Tables 4.7 and 4.8. IC_{50} values of the leaves and tubers extract for each cultivar were estimated and shown in Figures 4.1 and 4.2 respectively. IC_{50} is a standard measure of the efficacy and performance of a compound; it is the concentration of the

compound that results into 50% inhibition of a particular biological process (Sebaugh, 2011). IC_{50} value is inversely correlated with the radical scavenging activity of a compound. This means that, the lower the IC_{50} value, the stronger the antioxidant activity of the extracts. In the tubers, the aqueous extract of the cultivar 199062.1 had the lowest IC_{50} (3.90 ± 0.12 mg/ml). The aqueous extract of Bophelo and Impilo also had considerably low IC_{50} values (9.43 ± 0.53 mg/ml and 10.30 ± 0.93 mg/ml respectively) which were not significantly different from that of 199062.1. The highest IC_{50} values (30.16 ± 3.31mg/ml) were recorded in the aqueous-methanol extract of Blesbok. Although the aqueous-methanol extract of Bophelo had the lowest IC_{50} value among the leave extracts, the aqueous-methanol extracts of all the other cultivars had comparable IC_{50} values with no significant differences at $p<0.05$. The highest IC_{50} value was found in the aqueous extract of Monate. Generally, the orange fleshed cultivars showed lower IC_{50} values than the white fleshed cultivars. Furthermore, in the tubers, the aqueous extracts generally showed lower IC_{50} values than the aqueous methanol extracts, while in the leaves, the aqueous methanol extract showed lower IC_{50} values than the aqueous extracts.

Table 4.5: Antioxidant capacity (FRAP and TEAC) of tubers of seven South African grown cultivars of sweet potato.

EXTRACTS	CULTIVARS	FRAP (µmol AAE/g of extract)	TEAC(µmol TE/g of extract)
AQ	BLE	50.29 ± 1.12^{a}	12.45 ± 4.49^{a}
	BOP	222.60 ± 8.85^{b}	33.72 ± 1.70^{b}
	NDO	54.59 ± 1.75^{ah}	22.23 ± 0.64^{c}
	MON	163.90 ± 3.85^{c}	17.73 ± 6.32^{c}
	MUV	36.86 ± 0.85^{d}	18.40 ± 1.08^{c}
	IMP	145.70 ± 5.41^{ef}	13.63 ± 1.16^{a}
	199	165.70 ± 5.54^{c}	20.82 ± 5.46^{c}
AQ-ME	BLE	131.80 ± 5.01^{e}	17.75 ± 2.05^{c}
	BOP	158.60 ± 0.62^{f}	31.48 ± 1.75^{b}
	NDO	38.59 ± 2.35^{d}	19.62 ± 0.46^{c}
	MON	67.63 ± 4.03^{h}	26.58 ± 0.16^{b}
	MUV	12.55 ± 0.18^{g}	17.49 ± 0.74^{c}
	IMP	141.20 ± 13.83^{e}	28.02 ± 0.97^{b}
	199	24.01 ± 0.88^{dg}	20.14 ± 1.11^{c}

Values are mean ± SD (n=3). Same letters and different letters across the groups (down the vertical column) indicate no significant and significant difference respectively at $P<0.05$. BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract.

Table 4.6: Antioxidant capacity (FRAP and TEAC) of the leaves of seven South African grown cultivars of sweet potato.

EXTRACTS	CULTIVARS	FRAP (µmol AEE/g of extract)	TEAC(µmol TE/g of extract)
AQ	BLE	230.90 ± 12.74[a]	454.00 ± 7.04[a]
	BOP	335.70 ± 17.01[b]	581.40 ± 16.04[a]
	NDO	113.10 ± 6.43[c]	403.50 ± 1.10[a]
	MON	245.80 ± 8.31[a]	491.90 ± 9.67[a]
	MUV	109.50 ± 4.91[c]	278.30 ± 6.39[b]
	IMP	265.00 ± 5.80[a]	505.80 ± 10.09[a]
	199	220.00 ± 10.20[a]	592.50 ± 1.80[a]
AQ-ME	BLE	776.70 ± 14.44[d]	780.20 ± 3.92[c]
	BOP	844.10 ± 7.60[e]	812.30 ± 0.58[c]
	NDO	652.70 ± 12.42[f]	411.00 ± 8.61[a]
	MON	808.60 ± 30.98[e]	801.20 ± 10.26[c]
	MUV	605.70 ± 13.21[f]	678.30 ± 3.01[c]
	IMP	814.70 ± 32.04[e]	796.80 ±7.91[c]
	199	639.70 ± 17.20[f]	736.30 ± 4.62[c]

Values are mean ± SD (n=3). Same letters and different letters across the groups (down the vertical column) indicate no significant and significant difference respectively at $P<0.05$. BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract.

Table 4.7: Percentage inhibition (%) of Nitric oxide by tubers of seven South African grown cultivars of sweet potato.

CONC (mg/ml)	0.125		0.25		0.5		1	
CULTIVARS	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME
BLE	7.33 ± 0.49	5.5 ± 1.95	7.81 ± 1.94	7.34 ± 1.72	11.52 ± 3.66	12.96 ± 2.53	20.12 ± 0.92	16.09 ± 0.97
BOP	16.53 ± 1.21	5.08 ± 0.66	23.21 ± 1.42	6.19 ± 1.58	35.47 ± 0.86	13.18 ± 1.19	51.24 ± 2.57	21.62 ± 0.82
NDOU	7.81 ± 2.14	6.17 ± 2.23	13.76 ± 0.33	6.69 ± 1.00	18.67 ± 1.51	10.34 ± 4.27	25.82 ± 0.58	19.85 ± 1.89
MON	10.91 ± 1.6	8.22 ± 0.95	16.19 ± 1.78	10.74 ± 1.28	23.38 ± 3.64	17.21 ± 0.90	36.02 ± 1.04	22.04 ± 0.97
MUV	8.75 ± 1.67	7.07 ± 0.31	10.67 ± 3.32	7.75 ± 1.04	14.99 ± 1.61	12.15 ±2.23	26.26 ± 1.96	19.41 ± 0.94
IMP	16.2 ± 2.55	6.27 ± 0.14	19.46 ± 3.57	7.56 ± 0.24	31.77 ± 3.31	11.74 ± 1.7	48.74 ± 4.6	14.89 ± 1.74
199	27.67 ± 2.16	5.1 ± 0.17	47.86 ± 0.59	5.49 ± 0.21	63.79 ± 0.25	9.5 ± 0.25	77.58 ± 1.29	11.66 ± 0.46

Values are mean ± SD (n=3). BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract. CONC- Concentration.

Table 4.8: Percentage inhibition of Nitric oxide by leaves of seven South African grown cultivars of sweet potato.

CONC (mg/ml)	0.125		0.25		0.5		1	
CULTIVARS	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME
BLE	6.54 ± 0.73	6.27 ± 1.08	7.47 ± 1.46	7.03 ± 1.56	10.04 ± 1.65	13.29 ± 3.34	13.07 ± 1.43	25.68 ± 1.89
BOP	6.86 ± 1.04	5.11 ± 0.71	6.53 ± 2.47	10.25 ± 0.81	8.15 ± 3.65	18.03 ± 1.42	15.35 ± 1.11	32.78 ± 0.12
NDOU	3.76 ± 2.33	3.42 ± 0.27	4.72 ± 1.63	7.37 ± 1.3	6.51 ± 0.29	11.7 ± 3.38	11.55 ± 3.02	22.82 ± 1.41
MON	3.37 ± 1.25	4.05 ± 1.14	4.91 ± 1.88	8.13 ± 0.74	5.64 ± 1.65	13.72 ± 0.28	8.7 ± 2.58	26.67 ± 0.41
MUV	4.44 ± 1.44	5.06 ± 0.66	5.36 ± 0.46	8.35 ± 2.81	5.78 ± 2.38	12.76 ± 0.93	9.02 ± 2.72	22.35 ± 4.25
IMP	8.26 ± 0.16	2.84 ± 2.5	9.43 ± 0.41	10.11 ± 1.92	10.37 ± 0.14	15.39 ± 1.45	15.55 ± 0.42	29.07 ± 0.75
199	2.00 ± 0.49	7.21 ± 0.15	5.87 ± 0.98	9.35 ± 0.52	10.59 ± 1.63	17.39 ± 0.23	18.34 ±2.00	27.27 ± 1.2

Values are mean ± SD (n=3). BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, AQ, aqueous extract; AQ-ME, aqueous-methanol extract. CONC- Concentration.

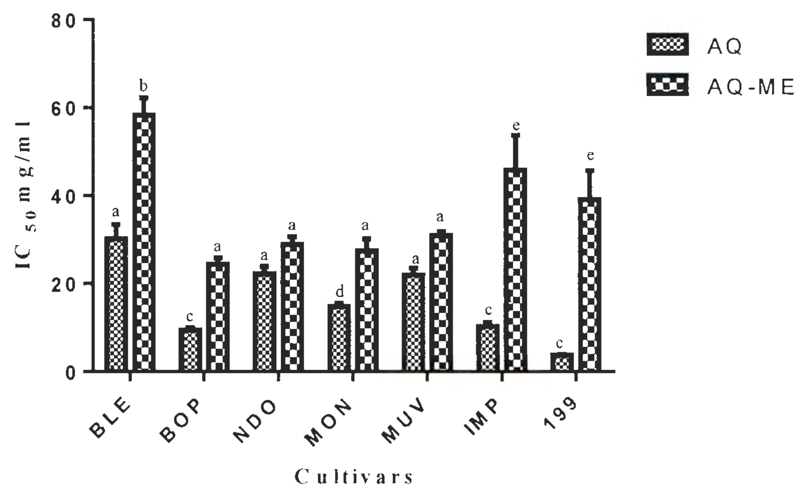


Figure 4.1: Nitric oxide scavenging activity IC50 value (mg/ml) of the tubers' extracts of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, A, aqueous extract; AQ-ME, aqueous-methanol extract.

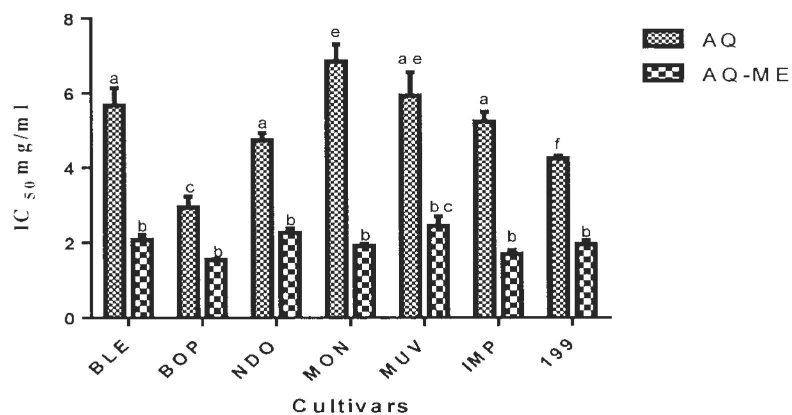


Figure 4.2: Nitric oxide scavenging activity IC50 value (mg/ml) of the leaves' extracts of seven South African grown cultivars of sweet potato. Values are mean ± SD (n=3). BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, A, aqueous extract; AQ-ME, aqueous-methanol extract.

4.3.3 Correlation analysis of total polyphenol contents and antioxidant activities

The correlation coefficient between the total polyphenol content and the antioxidant activity by the different assays were determined. Correlation analysis is used to determine the relationship between two quantitative variables (Gogtay *et al*., 2017). In this case, it was used to determine how much the total polyphenols content of the cultivars was able to influence their antioxidant activity. The correlation between the total polyphenol and the antioxidant activities are shown in Figures 4.3-4.5. There was a significant, strong and positive correlation between the polyphenol content and the antioxidant activities of the tubers and leaves measured by FRAP ($r = 0.81$, $r = 0.84$) and TEAC assays ($r = 0.71$, $r = 0.89$) respectively at $p<0.01$ (one-tailed). Several studies have demonstrated a strong correlation between polyphenol contents and antioxidant activities measured by FRAP and TEAC assays in different edible plants such as pomegranate (Fawole and Opara, 2016), apple (Xu *et al*., 2016), corn (Ku *et al*., 2014) as well as in sweet potato (Ghasemzadeh *et al*., 2012). A strong and significant correlation between total polyphenol and antioxidant activity by FRAP and TEAC methods indicates that polyphenols contributed largely to the antioxidant activity of the extracts. A significant negative correlation was found between the total polyphenol content and the IC50 values of the NO scavenging activity of the extracts at ($p<0.05$). However, while the correlation was moderate ($r = -0.59$) in the tubers, a strong correlation was recorded in the leaves ($r = -0.70$) extracts. Several studies have demonstrated a negative correlation between total polyphenol contents and IC_{50} values of Nitric oxide scavenging activity of plant extracts (Russo *et al*., 2015). The moderate correlation between the TPC of the leaves and the IC50 value of NO scavenging activity suggests that there are also other compounds which contribute to the scavenging activity of the extracts apart from polyphenols.

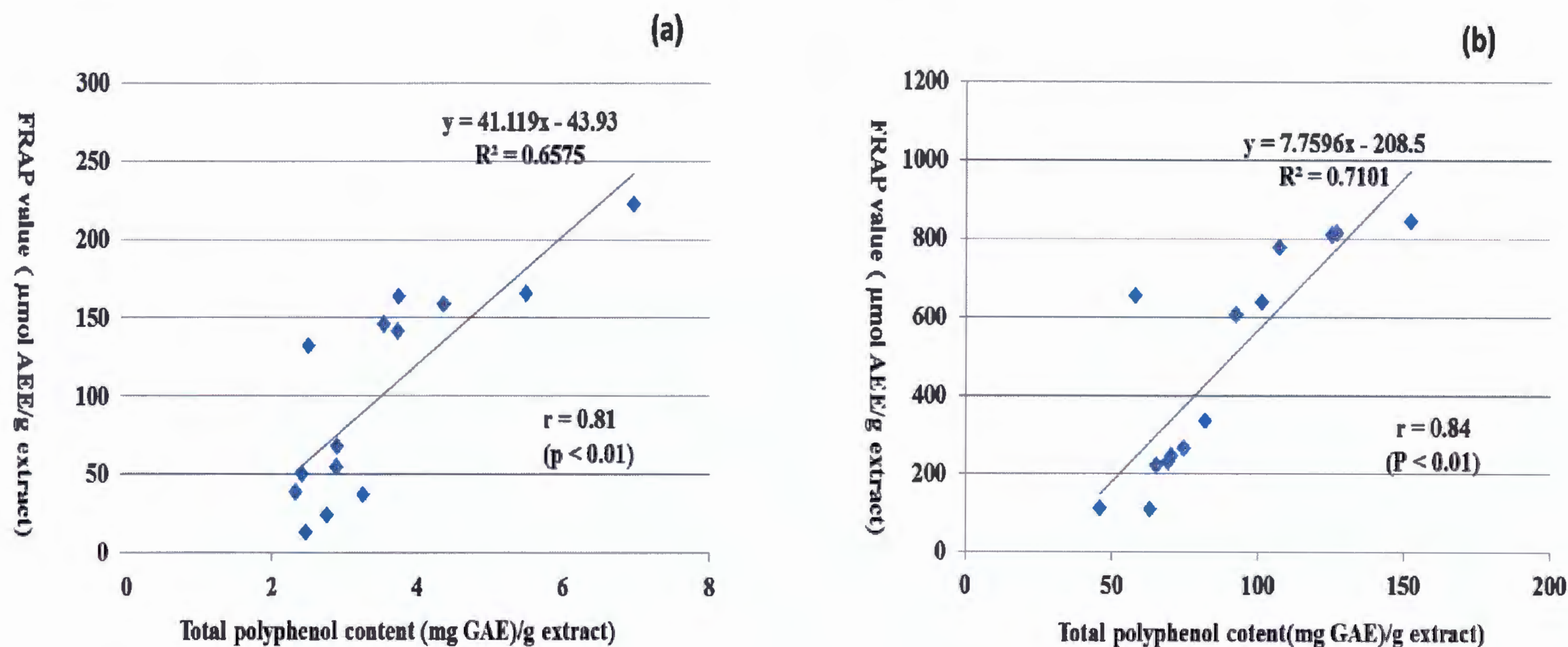
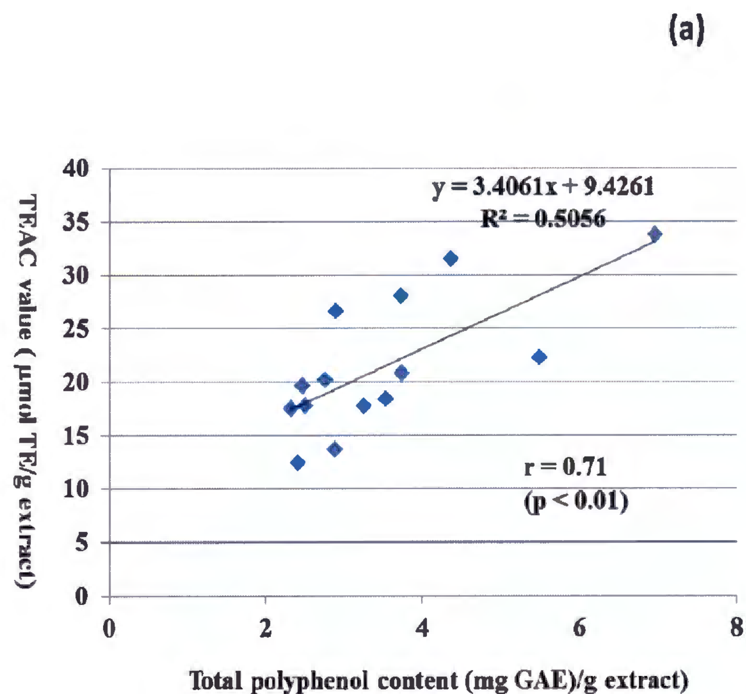


Figure 4.3: Correlation between total polyphenol content and FRAP values of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves. There were significant positive correlations of total polyphenol content in both the leaves and tubers with the FRAP values at ($P<0.01$).



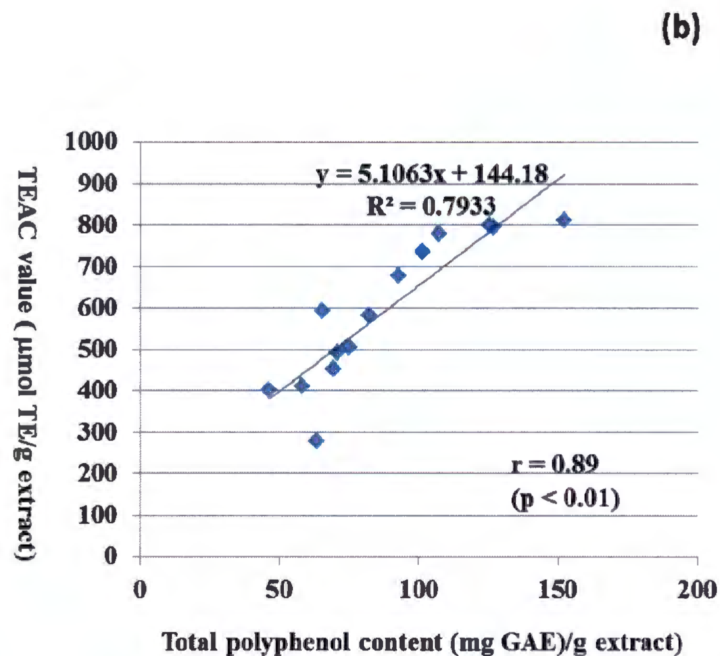


Figure 4.4: Correlation between total polyphenol content and TEAC values of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves. There were significant positive correlations of total polyphenol content in both the leaves and tubers with the TEAC values at ($P<0.01$).

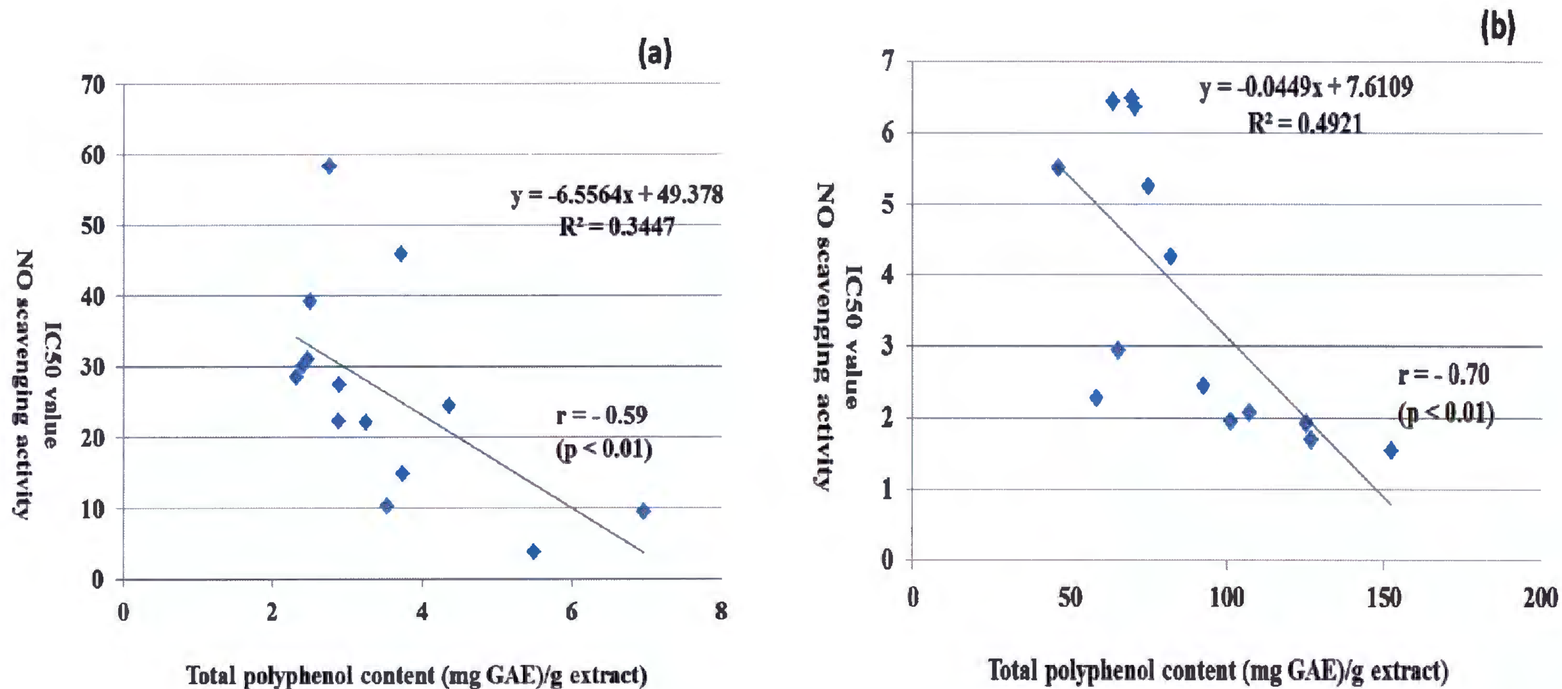


Figure 4.5: Correlation between total polyphenol content and IC_{50} values of Nitric oxide (NO) scavenging activity of aqueous and aqueous methanol extracts of seven South Africa grown sweet potato (a) tubers and (b) leaves. There were significant negative correlations of total polyphenol content in both the leaves and tubers with the FRAP values at ($P<0.01$).

4.3.4 Quantitative analysis of specific phenolic compounds and ascorbic acid

The level of specific phenolic compounds (caffeic acid, catechin, Hyperoside, Isoorientin, Isovanillic, kaempferol, orientin, protocatechuic acid, quercetin, rutin, vanyllic acid and vitexin) and ascorbic acid were also determined using LCMS. Analysis was performed using the multiple reaction monitoring (MRM) mode (Table 4.9). The peaks of compounds found in the extracts were confirmed by comparing the sample data to the retention time and liquid chromatography/mass spectrometry/mass spectrometry spectra of standards. The overlapped chromatograms of the standards and extracts are shown in figures 4.6 and 4.7 respectively. The individual chromatograms (addendum) showed the detection of the different compounds in the aqueous and aqueous-methanol extracts of both the leaves and tubers of the seven cultivars. The presence and concentration of the individual compounds vary from one cultivar to the other as shown in Table 4.10 (tubers) and 4.11 (leaves). With few exceptions, the leaves' extract had higher content of the phenolic compounds and ascorbic acid. The most abundant phenolic compound in the leave extracts was hyperoside with the highest levels (223.68 mg/g extract) found in the aqueous-methanol leave extract of Bophelo. Aqueous-methanol extracts of Blesbok, Monate and Impilo as well as both aqueous and aqueous-methanol extracts of 199062.1 also had considerable amount of hyperoside with values of 168.29 mg/g extract, 139.38 mg/g extract, 69.20 mg/g extract, 79.18 mg/g extract, 69.62 mg/g extract respectively. Hyperoside is a flavonoid compound which is commonly found in medicinal plants. Most Chinese herbal plants are rich in hyperoside which has been found to contribute significantly to the medicinal actions of these plants (Wang *et al.*, 2016). From literature, there have been little or no reports of the detection of hyperoside in sweet potato. This study has demonstrated the presence of hyperoside in the aqueous and aqueous-methanol extracts of both the leaves and tubers of sweet potato,

although, the amount is about 30 folds lower in the tubers. Caffeic acid is the most abundant compound in the tubers, the highest amount with a value of 19.40mg/gextract was found in the aqueous methanol extract of Bophelo. Aqueous methanol extracts of Impilo (16.42 mg/g extract) and Monate (13.38 mg/g extract) also had considerable amount of caffeic acid. In the leaves, a higher value (30.74mg/g extract) was found in the aqueous methanol extract of Blesbok. Several studies have reported the presence of caffeic acid in the leaves and tubers of various cultivars of sweet potato (Islam *et al.*, 2002; Harrison *et al.*, 2003; Teow *et al.*, 2007). Caffeic acid has been identified as a potent antioxidant agent in plants (Gulcin, 2006, Masek *et al.*, 2016). Olivier *et al.* (2006) reported the presence of caffeic acid in species of *Arctopus* and *Alepidea* which are important South African medicinal plants. Rutin and quercetin were also found in considerable amounts in most of the extracts especially in the aqueous methanol extracts of Bophelo cultivar which had the highest contents in the leaves (69.67, 37.76, mg/g extract) as well as in the tubers (2.91 and 1.76mg/g extract) extracts respectively. However, the amount of rutin and quercetin is negligible in the aqueous extracts of both the tubers and leaves of Bophelo cultivar. This further strengthens the importance of the influence of extraction solvents on the level of polyphenols found in plants. Ascorbic acid was also detected in the cultivars except in extracts of both the leaves and tubers of 199062.1 and the aqueous extracts of the tubers of Impilo. Kaempferol was not detected in the extracts of the cultivars except with few exceptions in the leaves extracts. This finding is similar to those of Ojong *et al.* (2008) and Park *et al.* (2016) where kaempferol was the least flavonoid detected in different varieties of sweet potato. Catechin, vitexin and orientin were also not detected in most of the extracts of both the leaves and the tubers of the sweet potato cultivars.

Table 4.9: Multiple reaction monitoring (MRM) transitions

Compound	Retention time (min)	Parent ion (m/z)	Product ion (m/z)	Dwell time	Fragmentor voltage	Collision energy
Rutin	13.7	611.2	303	200	129	21
Hyperoside	12.7	465.1	303.1	200	97	13
Orientin	13.1	449.1	329	200	160	33
Iso-orientin	13.8	449.1	299	200	129	29
Vitexin	13.6	433.1	313	200	160	33
Quercetin	15.7	303.1	153	200	160	41
Catechin	11.7	291.1	139	200	97	13
Kaempferol	15.8	287.1	167.2	200	160	49
Caffeic acid	12.4	181	163	200	65	9
Ascorbic acid	1.5	177	95	200	97	9
Isovanillic acid	12.6	169	65.1	200	97	25
VanIllic acid	12.5	169	65.1	200	97	29
Protocatechuic acid	7.9	155	65.1	200	97	21
2AAP	14.1	152.1	110	200	89	16

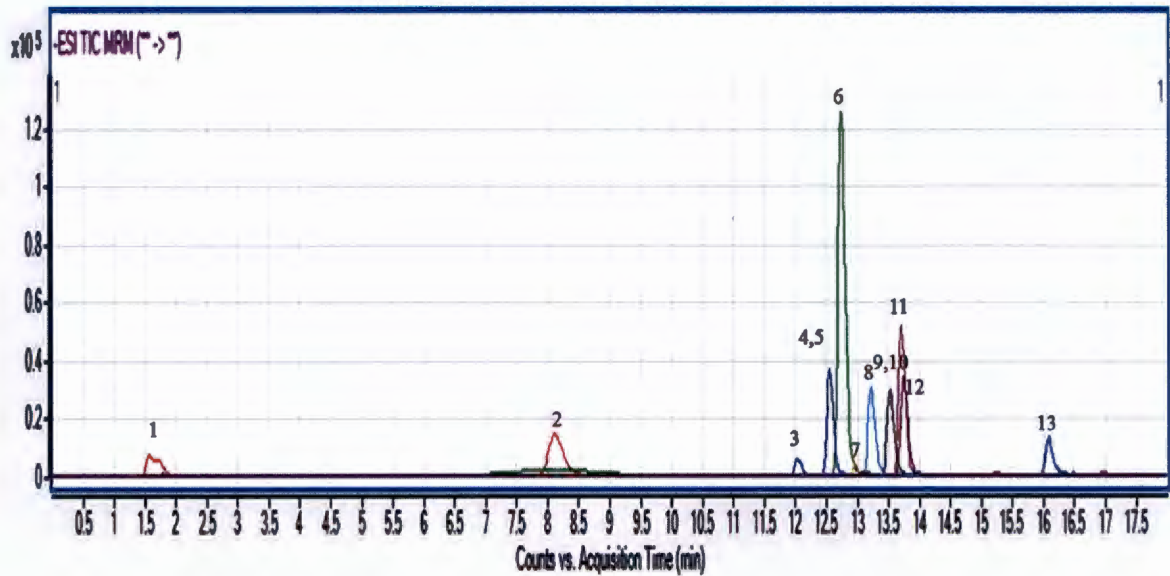


Figure 4.6: Overlapped chromatograms of standards polyphenols and ascorbic acid. 1- Ascorbic acid; 2- Protocathechuic acid; 3- Catechin; 4-Vanillic acid; 5-Isovanillic acid; 6- Hyperoside; 7-Caffeic acid; 8-Orientin; 9-Vitexin; 10- Rutin; 11-Isoorientin; 12-Quercetin; 13- Kaempferol

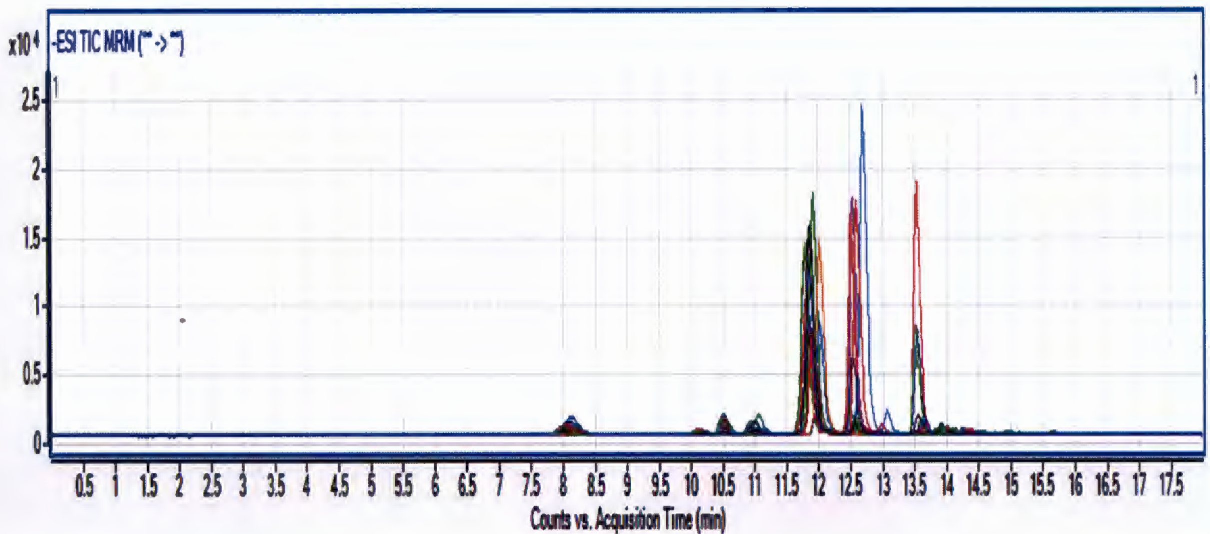


Figure 4.7: Overlapped chromatograms of aqueous and aqueous methanol extracts of the tubers and leaves of seven South African grown cultivars of sweet potato.

Table 4.10: Phenolic compounds and ascorbic acid composition of tubers of seven South African grown cultivars of sweet potato.

	CULTIVARS													
	BLE		BOP		NDO		MON		MUV		IMP		199	
COMPOUNDS (µg/mg)	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME	AQ	AQ-ME
Caffeic acid	0.17	1.05	1.70	19.40	0.16	0.51	0.58	13.38	0.02	0.63	6.85	16.42	0.00	0.62
Catechin	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND	0.06	ND	ND	ND
Hyperoside	0.28	2.06	0.22	9.78	0.22	1.72	0.22	1.91	0.27	1.18	0.16	2.05	0.19	0.71
Iso-orientin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.11	ND	ND
Kaempferol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Quercetin	ND	0.34	0.03	1.76	ND	0.33	0.05	0.40	0.04	0.23	0.04	0.30	0.03	0.10
Rutin	0.08	2.21	ND	2.91	ND	0.33	0.06	0.61	0.05	0.21	0.25	1.60	0.10	0.18
Orientin	ND	ND	ND	ND	ND	ND	ND	0.03	ND	ND	ND	ND	ND	ND
Vitexin	ND	ND	ND	ND	ND	ND	0.01	0.02	ND	ND	ND	ND	ND	ND
Protocatechuic acid	1.40	1.65	0.25	0.08	0.53	0.08	2.72	0.12	0.85	0.08	1.38	0.62	1.84	0.24
Vanyllic acid	0.62	1.04	0.45	0.72	0.77	0.95	0.69	0.73	0.47	0.41	0.60	0.65	0.16	0.15
Isovanillic acid	0.02	ND	0.14	0.45	0.22	0.43	0.32	0.37	0.37	0.32	0.15	0.18	0.20	0.57
Ascorbic acid	0.10	0.18	0.09	0.12	0.12	0.13	0.22	0.26	ND	0.36	0.14	ND	ND	ND

BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, A, aqueous extract; AQ-ME, aqueous-methanol extract; ND, Not detected.

Table 4.11: Phenolic compounds and ascorbic acid composition of leaves of seven South African grown cultivars of sweet potato.

	CULTIVARS													
	BLE		BOP		NDOU		MON		MUV		IMP		199	
COMPOUNDS(µg/mg)	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM	A	AM
Caffeic acid	0.57	30.74	0.98	3.76	0.27	0.31	1.57	0.84	0.70	0.69	0.99	1.33	1.57	1.15
Catechin	0.09	0.08	ND	0.02	0.06	0.05	0.16	ND	ND	0.16	0.05	ND	0.06	0.05
Hyperoside	0.36	168.29	1.39	223.68	1.50	27.80	5.00	139.38	1.60	23.38	2.29	69.20	79.18	69.62
Iso-orientin	ND	0.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.14	0.27	0.19
Isovanillic acid	ND	0.04	0.04	0.13	0.03	0.07	0.04	0.07	0.04	0.07	0.05	0.12	0.13	0.11
Kaempferol	ND	0.16	ND	0.09	ND	0.06	0.02	0.28	0.01	ND	ND	0.10	0.10	ND
Orientin	ND	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.18	0.12
Protocatechuic acid	1.01	2.03	0.45	1.22	0.68	0.93	0.53	1.33	0.41	0.88	0.50	0.89	0.72	0.42
Quercetin	0.06	27.87	0.28	37.76	0.26	4.73	0.87	23.36	0.31	4.06	0.41	11.97	14.58	12.85
Rutin	ND	9.60	0.73	69.67	ND	1.84	0.59	17.18	0.18	3.09	0.74	24.95	28.14	24.73
Vanyllic acid	0.04	0.59	0.26	0.40	0.35	0.80	0.43	0.53	0.36	0.62	0.29	0.66	0.67	0.55
Vitexin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.26	0.35	0.33
Ascorbic acid	0.11	0.10	0.11	0.15	0.13	0.13	0.10	0.13	0.10	0.21	0.09	0.14	ND	ND

BLE, blesbok; BOP, bophelo; NDO, ndou; MON, monati; MUV, Mvuvhelo; IMP, impilo; 199, 199062.1, A, aqueous extract; AQ-ME, aqueous-methanol extract; ND, Not detected.

4.4 CONCLUSION

All the cultivars used in this study contained polyphenols and showed antioxidant activities but with variations across the cultivars. Generally, the orange fleshed cultivars mostly had higher antioxidant profiles and showed stronger antioxidant activity than the white fleshed cultivars in both the leaves and the tubers. The higher content of antioxidant profiles in the orange fleshed cultivars suggests that they are excellent source of beneficial polyphenols. Bophelo, an orange fleshed cultivar, consistently showed higher concentrations of antioxidant profiles and antioxidant activities with few exceptions. There were significant correlations between the total polyphenol contents and antioxidant activities which suggest that the stronger antioxidant activities displayed by the Bophelo cultivar are mostly due to its higher phenolic content. Furthermore, the leaf extracts also generally contained higher concentrations of antioxidants and also showed stronger antioxidant activity than the tubers. Solvent is another important factor which influences the outcome of this study. Given that sweet potato is mostly consumed as tubers, the introduction of the leaves into diet, especially in the rural areas, can provide access to cheap dietary antioxidants. The cultivation and consumption of orange fleshed sweet potato cultivars should also be more encouraged not only for its higher vitamin A content, but also for its high level of antioxidant compounds. The extracts of 'Bophelo' were further used in this study to explore its antioxidant activity and potential therapeutic effects on oxidative stress mediated insulin resistance.

CHAPTER FIVE

EXTRACTS OF ORANGE SWEET POTATO AMELIORATE OXIDATIVE STRESS AND UPREGULATE DIABETES RELATED AND ANTIOXIDANT GENES IN INSULIN RESISTANT C2C12 CELLS.

ABSTRACT

Insulin resistance is a hallmark of type 2 diabetes, especially in the skeletal muscle. Antioxidant therapy is an emerging strategy in the management of insulin resistance due to the increasing evidences of the involvement of oxidative stress in its pathology. Therefore, edible plants which are sources of natural antioxidants can be utilized in the management and treatment of insulin resistance. The antioxidant activity of sweet potato extracts, especially the orange fleshed cultivars, has been demonstrated in many studies. Orange fleshed sweet potatoes are particularly rich in carotenoids and polyphenols which are strong antioxidants. The antioxidant activity of Bophelo, an orange fleshed sweet potato cultivar has been demonstrated in a previous study. This study, therefore, sought to investigate the effect of extracts of 'Bophelo' on oxidative stress in palmitate-induced insulin resistance in C2C12 cells. It went further to determine the effects of the extracts on the regulation of some antioxidant enzymes as well as diabetes related genes in the C2C12 skeletal muscle cells. Palmitate treated and healthy C2C12 cells were treated with 500μg/ml and 100μg/ml of the tubers' and leaves' extracts respectively. Treatment with 1μM of metformin was used as a positive control. Expression level of diabetes related genes (GLUT4, NRFI, MEF2A, CPT1 and ACC2) and antioxidant genes (GPX1 and CAT) were analyzed by Real Time Quantitative Polymerase Chain Reaction (RT-qPCR). Oxidative stress biomarkers which include total glutathione status (GSH), glutathione peroxidase (GPx) activity, lipid peroxidation (LPO) and catalase (CAT) activity were determined using established techniques.

Antioxidant status of the cells were also determined using FRAP and TEAC assays. There were significant decreases in expression of GLUT4, NRF1 and MEF2A in the palmitate treated group which increased significantly upon treatment with the extracts and metformin. There was also a non-significant ($P>0.05$) increase in expression of CPT1, but a significant ($P<0.05$) decrease in the expression level of the ACC2 gene in the insulin resistant cells treated with sweet potato extracts and metformin. The genes of both antioxidant enzymes (CAT and GPx) were also significantly decreased in the palmitate induced insulin- resistant cells and was upregulated after treatment with the extracts and metformin. Furthermore, oxidative stress assays showed a significant increase and reduction in the total glutathione status and level of malonaldehyde (MDA) respectively in the insulin resistant C2C12 cells upon treatments with extracts and metformin. On the other hand, GPx activity increased significantly ($P<0.05$) while catalase activity reduced, although not significantly ($P>0.05$) in the palmitate treated groups. These were also improved significantly upon treatment with the extract and metformin. FRAP and TEAC values also reduced in the palmitate treated groups and improved significantly ($P<0.05$) following treatment with the extracts and metformin. Healthy C2C12 cells treated with extracts only also improved across all the parameters measured.

5.1 INTRODUCTION

Insulin resistance is generally an impaired ability of insulin to stimulate glucose uptake and utilization (Defronzo and Tripathy, 2009). It is the most powerful indicator of an imminent development of type 2 diabetes and therefore an important therapeutic target in the management of the disease (Taylor, 2012). Oxidative stress which occurs as a result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses is a major pathological cause of several chronic diseases including diabetes (Morales-González, 2013). Oxidative stress has been implicated in the development of insulin resistance and its progression into type 2 diabetes and its complications (Keane *et al.*, 2015; Tangvarasittichai, 2015). Therefore, the inhibition of oxidative stress is crucial in the prevention and management of insulin resistance and diabetes.

Natural antioxidants from edible plants are a source of dietary components that can act as reducing agents against these reactive oxygen molecules and thus promote a healthy life. Orange sweet potato cultivars are particularly rich in carotenoids and polyphenols both of which are strong antioxidants (Alam *et al.*, 2016). Extracts of 'Bophelo', an orange-fleshed sweet potato showed the strongest activity in a prior study comparing the antioxidant ability of seven different South African cultivars of sweet potato. Our findings from the previous study also showed that the aqueous-methanol extracts of the leaves of 'Bophelo' cultivar had higher concentration of antioxidant profiles than the aqueous extracts. On the contrary, the aqueous extracts of the tubers mostly showed higher concentration and stronger antioxidant activity than the aqueous-methanol extracts. This work, therefore, investigated the effects of aqueous extracts of the tubers and aqueous-methanol extracts of the leaves of 'Bophelo' on some markers of oxidative stress and expression level of antioxidant enzymes in insulin resistant C2C12 cells.

Furthermore, an important factor involved in the development of insulin resistance is the reduced ability of skeletal muscle to adjust easily between glucose and fatty acid oxidation in response to homeostatic signals (Cahova *et al.*, 2007). This makes glucose and fatty acid metabolisms important metabolic processes involved in the pathophysiology of insulin resistance and diabetes. Therefore, another strategy in the management of type 2 diabetes is the modulation of the genes and transcription factors involved in glucose and fatty acid metabolism. In this perspective, this study also assessed the effects of the extracts on the expression level of glucose transporter 4 (GLUT4), nuclear respiratory factor 1 (NRF1), myocyte enhancer factor 2A (MEF2A), carnitine palmitoyltransferase 1 (CPT1) and acetyl-CoA-carboxylase 2(ACC2) in C2C12 cells. GLUT4 is a key glucose transporter which plays an important role in the maintenance of glucose homeostasis in the body and thus an important pharmacological target in the management of glucose homeostasis in type 2 diabetes (Henriksson and Zierath, 2001; Huang and Czech, 2007). MEF2A and NRF1 are transcription factors that both play important roles in the regulation of GLUT-4 expression and ultimately glucose uptake metabolism (Mcgee and Hargreaves, 2006; Anderson *et al.*, 2015). ACC2 and CPT1 are actively involved in fatty acid synthesis and oxidation as well as their interrelations with glucose utilization (Wakil and Abu-Elheiga, 2009). The change in expression of these genes in insulin-resistant C2C12 cells treated with sweet potato extracts, in comparison with untreated cells, will shed some light on the molecular mechanisms behind the hypoglycemic activity of sweet potato.

5.2 MATERIALS AND METHODS

5.2.1 Cell culture

Cell culture activities were performed, maintained and monitored in a class 2 biological safety cabinet (ESCO), Forma® Series II Water Jacketed CO2 Incubator (37°C and 5% CO_2) and EVOS FL digital microscope (Life Technologies) respectively. The base medium used for all cell culture was Dulbecco's Modified Eagle's Medium - high glucose (DMEM) (Sigma-Aldrich) containing 4500mg/L glucose and L-glutamine and supplemented with 3.7g/L $NaHCO_3$ and 1.5g/L creatine.

5.2.1.1 Storage of cell lines

C2C12 cells from (ATCC) were stored and maintained in freezing media containing 70% DMEM, 20% FBS and 10% DMSO in -80°C and liquid nitrogen.

5.2.1.2 Thawing and proliferation of C2C12 cells

Cryo vial containing C2C12 cells was removed from liquid nitrogen and thawed briefly in 37°C water bath. Cells were transferred into 15ml tube containing 10ml of pre-warmed growth medium (89% Dulbecco modified eagles medium (DMEM), 10% FBS and 1% antibiotics) and centrifuged at 2000rpm for 3 minutes. The supernatant was decanted and cells were re-suspended in 10 ml of pre-warmed growth media and transferred into 100mm TC dish. The dish was placed into the incubator and the growth media was changed every 1-2 days as necessary.

5.2.1.3 C2C12 cells subculture and differentiation

In order to get adequate cells for the experiments, C2C12 cells from the original dish at about 70% confluence were sub-cultured into three 100mm TC dishes. The growth media in the

original dish was sucked off and the cells were washed with 10mls of pre-warmed PBS twice to adequately remove the serum. Cells were lifted from the plate by adding 1ml of 0.25% trypsin (Sigma) and incubating at 37°C for 5-10 minutes until cells are rounded up and detached from the plate. Trypsinization was monitored under the microscope and stopped by adding 2mls of growth media to the cells. The suspended cells were then transferred into a 15mls tubes and centrifuged at 2000rpm for 3 minutes. The medium was decanted and the cells were re-suspended in 1ml of growth media. The density of the cells in the media was estimated using a TC 20 automated cell counter (BioRad). Appropriate volume of media containing approximately 2.2×10^6 were plated in 100mm petri dishes containing 10mls of growth media. At about 70% confluency, C2C12 cells were differentiated for 4 days by adding differentiation media containing 97% DMEM, 2% FBS and 1% antibiotics. The media was changed every 24 hours until formation of myotubes were observed.

5.2.2 Preparation of sweet potato samples and extracts

Orange fleshed sweet potato cultivar 'Bophelo' is a product of Agricultural Research Council (ARC), South Africa and was cultivated by the Department of Crop Science, North West University, Mafikeng campus, South Africa. The leaves and root tubers were harvested fresh from the university farm. They were rinsed under running water; the leaves were cut into pieces and tubers into thin chips before being air dried in the laboratory at room temperature (25 ± 2°C). Air dried samples were ground into fine powder (about 500µm mesh size) and packed in an air tight container. They were labelled accordingly and stored at 4°C for further use. Preparation of crude aqueous extracts of the tubers and aqueous-methanol (1:1) extracts of the leaves were done by soaking the powder in the respective solvents (5g/100mls) for 24 hours at room temperature with constant shaking. Extracts were recovered through lyophilization and evaporation using a

freeze dryer (Alpha 1-4 LSC Plus) and a rotary evaporator (RE-52A) respectively. Dried aqueous extracts of the tubers (OSPT) and aqueous-methanol extracts of the leaves (OSPL) were stored in the dark at -20°C until further use.

5.2.3 MTT cytotoxicity assay

MTT assay was carried out using the Vybrant® MTT Cell proliferation assay kit (Molecular Probes). The principle of MTT assay involves the conversion of water soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into an insoluble product. Living and viable cells with active metabolism are able to convert MTT into a purple coloured formazan. The quantity of formazan is considered to be directly proportional to the number of viable cells and is measured after being solubilized in a buffer by recording changes in absorbance at 570 nm with a spectrophotometer (Riss *et al.,* 2016). C2C12 cells were plated at a density of 5×10^4 in a 96 well plate. After 48 hours differentiation, cells were treated with different concentrations (10, 20, 50, 100, 200, 500, 1000µg/ml) of OSPT and OSPL in growth medium for 24 hours at 37°C. Thereafter, the media containing the extracts were removed and replaced with 100µl of fresh medium and 10µl of 12mM MTT solution in sterile PBS. Control sample containing untreated cells labelled with MTT and sample blank containing only medium (without cells) and MTT were also included. The plate was incubated at 37°C for 4 hours, 50µl of DMSO was then added to the wells and mixed thoroughly to dissolve the precipitate formed. The absorbance of the wells was read at 570nm after incubation at 37°C for 10 minutes. Cell viability was expressed as percentage and estimated as;

$$Absorbance\ of\ sample\ -\ absorbance\ of\ sample\ blank\ /\ Absorbance\ of\ control\ -\ Absorbance\ of\ sample\ blank\ \times\ 100$$

5.2.4 Preparation of palmitate and induction of insulin resistance

Palmitate was prepared in 50% ethanol solution heated to 95°C (Mazibuko *et al.*, 2013). Prior to treatment with palmitate, cells were serum and glucose starved by incubating in PBS for 30 minutes at 37°C and in 5% CO_2. Insulin resistance was induced by incubating the cells with DMEM containing 2% BSA and 0.75mM palmitate for 16 hours.

5.2.5 Treatment of C2C12 cells with sweet potato extracts

Fresh stock solutions of OSPT and OSPL (50mg/ml and 5mg/ml respectively) were prepared in sterile distilled water and filtered through a 0.20µm syringe filter. Prior to treatment, after washing the cells with PBS, they were incubated for 30 minutes also in PBS to starve them of glucose and serum. Cells were divided into seven groups and treated according to Table 5.1. Differentiated C2C12 cells were treated with 500µg/ml and 100µg/ml of OSPT and OSPL respectively in base DMEM supplemented with 2% BSA for 3 hours. Untreated cells served as negative control while cells treated with 1µM metformin served as positive control. Treatment with only OSPT and OSPL were included to assess the protective effect of the extracts on healthy C2C12 skeletal muscle cells.

5.2.6 Gene expression analysis

On the day of experiment, cells were washed with PBS and harvested by scraping the cells with growth medium into RNase free Eppendorf tubes and kept on ice prior to extraction of RNA.

5.2.6.1 Total RNA extraction and purification

Total RNA extraction from the cells and purification was carried out using PureLink® RNA mini kit and according to the manufacturer's manual. The tubes containing the harvested cells were

centrifuged at 2000g for 5 minutes at 4^oC to collect the cells. The growth medium was discarded and 600μl of lysis buffer containing 0.2% mercaptoethanol was added to the cells and vortexing of the mixture was done until cell pellet was completely dispersed. The cell lysate was homogenized with a Stuart® SHM1 homogenizer at the maximum speed for 60 seconds. The homogenate was centrifuged at 26000g for 5 minutes and the supernatant was transferred into a clean RNase free tube. To purify the RNA, 600μl of 70% ethanol was added to the homogenates and the mixture was vortexed thoroughly until there were no visible precipitates in the tubes. The homogenates were transferred into spin cartridges inserted into collection tubes and were centrifuged at 12,000g for 15 seconds at room temperature. The flow through was discarded and 700μl of 'wash buffer 1' was added into the spin cartridges and centrifuged for another 15 minutes at 15,000g. The flow through was again discarded and the spin cartridges were inserted into new collection tubes and 500μl of 'wash buffer 2' containing ethanol was added to the spin cartridges which were centrifuged at 15,000g for 15 seconds at room temperature. The addition of 'wash buffer 2' and centrifugation was repeated. The spin cartridges were centrifuged at 12,000 g for 2 minutes to dry the membrane of the spin cartridges with bound RNA. Elution of RNA was done by adding 50μl of RNase free water to the center of the cartridges, incubation for 1 minute at room temperature and centrifugation at 12,000g for 2 minutes. The yield of RNA was quantified using a nanodrop spectrophotometer.

5.2.6.2 Agarose Gel electrophoresis

The integrity of the RNA was confirmed by loading the RNA samples on 1% agarose gel. 0.5g agarose was added to 50 ml of 1x Tris Acetate EDTA (TAE) buffer, the mixture was heated at medium temperature in a microwave for 2 minutes to dissolve the agarose. The agarose solution was allowed to cool down to about 60^oC and 2.5μl of 5μg/μl ethidium bromide was added. The

mixture was poured into a gel cast tray carefully to avoid bubbles. Comb was inserted into the agarose and allowed to stand at room temperature for about 30 minutes to allow for solidification. The solidified agarose was transferred into the electrophoresis unit which was then filled with 1x TAE buffer until the gel was completely covered. One microliter (1µl) of 6x gel loading dye was added to 5µl of the RNA sample. One kb molecular weight ladder (500-10,000 bp) was loaded on the first lane of the gels while the samples were loaded in other wells accordingly. The gel was run at constant voltage of 90V for 1 hour after, it was removed carefully and the bands were visualized using a Gel Doc™ system (BioRad).

5.2.6.3 Complimentary DNA (cDNA) synthesis

The extracted mRNA was reverse-transcribed into first-strand cDNA using a Superscript™ VILO™ Mastermix. 4µl of mastermix was added to a volume of RNA equivalent to 2.0µg of total RNA and was made up to 20µl volume with DEPC treated water in small PCR tubes according to Table 5.2. Mixing was done by pipetting gently up and down in tubes and incubation was done using a thermal cycler programmed at 25°C for 10 minutes, 42°C for 60 minutes and the terminating reaction at 85°C for 5 minutes. The synthesized cDNA was stored in -20°C for onward use in qPCR reactions.

Table 5.1: Treatment groups for C2C12 cells

Treatment groups	Palmitate (PT)	Tuber extracts (OSPT)	Leaf extracts (OSPL)	Metformin (M)
CONTROL	-	-	-	-
PT	+	-	-	-
PT+OSPT	+	+	-	-
PT+OSPL	+	-	+	-
OSPT	-	+	-	-
OSPL	-	-	+	-
PT+M	+	-	-	+

+, treated with; -, not treated with

Table 5.2: Yield of extracted RNA from the treatment groups and the reaction components for the synthesis of cDNA

	CONTROL	PT	PT+OSPT	PT+OSPL	PT+M	OSPT	OSPL
Yield (ng/µl)	494.7	238.9	337.8	475.6	495.8	468.8	496.9
Superscript™ VILO™ mastermix	4.0 µl	4.0 µl	4.0 µl	4.0 µl	4.0 µl	4.0 µl	4.0 µl
RNA(2.0µg)	4.0 µl	8.4 µl	5.9 µl	4.2 µl	4.0 µl	4.3 µl	4.0 µl
DEPC-treated water	12.0 µl	7.6 µl	10.1 µl	11.8 µl	12.0 µl	11.7 µl	12.0 µl
Total Volume	20µl	20µl	20µl	20µl	20µl	20µl	20µl

5.2.6.4 Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

Real Time qRT-PCR was carried out using a Steponeplus^{TM} PCR machine (Applied Biosystems) and the PowerUp^{TM} SYBR^{TM} Green Master Mix (Applied Biosystem). The genes of interest (GOI) are; glucose transporter 4 (GLUT4), nuclear receptor factor 1(NRF1), myocyte enhance factor 2A (MEF2A), carnitine palmitoyl transferase 1(CPT1), acetyl CoA carboxylase 2(ACC2), glutathione peroxidase (GPx) and catalase (CAT). GAPDH was used as reference gene. The reaction mix of the qRT-PCR reactions (Table 5.3) contains approximately 10ng (2µl) of cDNA from each treatment group and per each gene of interest (GOI) and the reference gene (RG). Specific primers (forward and reverse) designed for amplification of the genes (Table 5.4) were also included in the reaction mix. No template control (NTC) reactions (no cDNA in reaction mix) were also set up to detect PCR contaminations. The components were mixed thoroughly by pipetting up and down carefully to avoid bubbles. The reaction mix was then pippeted in triplicates into the PCR plate and loaded on to the steponeplus^{TM} qRT-PCR machine according to the layout in Figure 5.1. The reaction volume on the machine was set to 10µl and thermal cycling conditions were programmed to Uracil-DNA-glycosylase (UDG) activation (50°C for 2 minutes, hold), initial denaturation (95°C for 2 minutes, hold); denaturation (95°C for 15 seconds, 40 cycles); annealing and extension (60°C for 1 minute, 40 cycles).

Relative gene expression was analyzed by the comparative ΔCT method by using the formula, $2^{-\Delta\Delta CT}$. The expression level of the gene of interest is presented as relative expression, that is, relative to a reference gene (GAPDH) and as a fold change in expression level relative to the control treatment.

Table 5.3: Component mix for Quantitative reverse transcription polymerase chain (qRT-PCR) reaction

COMPONENT	VOLUME
PowerUpTM SYBRTM Green Master Mix	5 µl
Forward primer	1 µl
Reverse primer	1 µl
cDNA	2 µl
DEPC-treated water	1 µl
Total	10 µl

Table 5.4: Primers sequences for Quantitative reverse transcription polymerase chain (qRT-PCR) reaction

Gene	Primer sequence(5´-3´)
GLUT-4	Forward - AAGATGGCCACGGAGAGA
	Reverse - GTGGGTTGTGGCAGTGAGTC
NRF-1	Forward - AAACACAAACTCAGGCCACC
	Reverse- CCATCAGCCACAGCAGAGTA
MEF-2A	Forward - GTGTACTCAGCAATGCCGAC
	Reverse - AACCCTGAGATAACTGCCCTC
CPT-1	Forward - CCAGGCTACAGTGGGACATT
	Reverse - GAACTTGCCCATGTCCTTGT
ACC-2	Forward - GTCCTCATCATGAACGGCTG
	Reverse -AGGACAGTGGGGTCGTTTTC
GPX	Forward - CAGTCCACCGTGTATGCCTT
	Reverse - GTAAAGAGCGGGTGAGCCTT
CAT	Forward - CGGGCCTGGCCGATG
	Reverse - GCCATTCATGTGCCGATGTC
GAPDH	Forward - GCACAGTCAAGGCCGAGAAT
	Reverse - GCCTTCTCCATGGTGGTGAA

5.2.7 Oxidative stress and antioxidant activity assays

Prior to each assay, protein determination was carried out on the cell homogenates for normalization across the treatment groups.

5.2.7.1 Protein determination assay

Bradford protein assay was carried out to normalize the assessment of the level of oxidative stress and antioxidant activity across the treated cells. Cells were seeded into 6-well plates at a density of 0.3×10^6. Differentiated cells were treated as described in section 5.2.5 and harvested by scraping with sterile PBS into Eppendorf tubes. The cells were then homogenized on ice for about 2 minutes. The homogenates were centrifuged at 4^oC for 15 minutes. The supernatants were removed and kept on ice for use in the assay. Five microliters (5µl) of the supernatants or protein standards (0.2-1.0mg/ml) followed by 250µl of Bradford reagent was added in triplicates to the wells of 96 well plate, the absorbances of the samples were read at 595nm after incubation for 5 minutes. The net absorbance of the standards was plotted against the concentrations to generate a standard curve. The protein concentrations of the samples were extrapolated from the standard curve.

5.2.7.2 Total glutathione status and Glutathione peroxidase activity assays

These were carried out according to the method of Rotruck *et al.* (1973) using reduced glutathione as standard. 1mM stock solution of reduced glutathione was prepared in 0.2M EDTA (pH 8). This was further diluted in EDTA solution to generate 25, 50, 100, 150, 200µM of reduced glutathione standard solutions. 100µl of 0.3M K_2HPO_4 and 50µl of 0.04% DTNB was added to 50µl of the cell homogenate or standards. Absorbance of the reaction mixture was read at 412nm against a blank and the concentrations of the standards were plotted against the

NWU
LIBRARY

absorbances. Total glutathione of the samples were estimated from the generated standard curve. To estimate the glutathione peroxidase activity, a reaction mixture according to Table 5.5 was prepared. The mixture was incubated at 37°C for 3 minutes followed by the addition of 0.5ml of 10% TCA and centrifugation at 3000rpm for 5 minutes. 100µl of 0.3M K_2HPO_4 and 50µl of 0.04% DTNB was added to 50µl of the supernatant or standards. Absorbance of the reaction mixture was read at 412nm against a blank. The concentration of the remaining GSH was extrapolated from the standard curve and glutathione peroxidase activity was calculated by the formula;

$$GSH\ consumed = 245.34 - GSH\ remaining$$

$$Glutathione\ peroxidase\ activity = Amount\ of\ GSH\ consumed/mg\ protein$$

Glutathione peroxidase activity was expressed as µg of GSH consumed/mg of protein

5.2.7.3 Catalase activity assay

Catalase activity was measured by the method of Sinha (1972) and Hadwan (2016). The principle is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide. The assay was carried out as described in Table 5.6, the mixture was vortexed and incubated for 3 minutes after which there was addition of 2mls of dichromate/acetic acid to each test. Thereafter, the tubes were kept at 100°C for 10 minutes and centrifuged at 2500g for 5 mins after cooling to remove precipitated protein. Changes in absorbances were recorded at 570nm against the blank sample. Catalase activity was calculated by the equation: $2.303/t \times \{log\ So/S - M\} \times Vt/Vs$

where t: time taken for the reaction

S^o: Absorbance of standard tube

S: Absorbance of test tube

M: Absorbance of control test

Vt: Total volume of reagents in test tube

Vs: Volume of serum

Table 5.5: Reaction mixture for glutathione peroxidase activity

Reagents	Volume (µl)
Potassium Phosphate buffer (100mM, pH 7.5)	500
NaN_3 (10mM)	100
GSH(4mM)	200
H_2O_2 (2.5mM)	100
Distilled H_20	500
Sample	600

Table 5.6: Reaction mixture for catalase activity assay

Reagents	Sample	Control	Standard	Blank
Sample	100µl	100µl	-	
Distilled H_2O	-	1000µl	100µl	1100µl
H_2O_2	1000µl	-	1000µl	

5.2.7.4 Lipid peroxidation assay

The extent of lipid peroxidation across the treatment groups were measured using the thiobarbituric acid reactive substances (TBARS) assay as described by Buege and Aust (1978). The principle is based on the reaction between the end product of lipid peroxidation, malondialdehyde (MDA) and (TBA) to produce a red product that can be measured spectrophotometrically (Garcia *et. al*., 2005). 200μM stock solution of the standard (MDA) was prepared in phosphate buffer saline and further diluted to generate 2, 4, 6, 8, 10μM of MDA solution. 100μl of cell homogenates/standards were added to 1ml of 0.67% (w/v) TBA, 1ml of 20% (w/v) trichloroacetic acid (TCA) and 1.5ml of 0.04% butylated hydroxytoluene (BHT) in microtubes. The mixture was incubated in boiling water for 20 minutes and then cooled to room temperature. Thereafter, the tubes were centrifuged at 4,000g for 10 minutes, the supernatants were collected and the changes in absorbances were measured at 532nm against blank. The concentrations of MDA were extrapolated from the standard curve obtained by plotting the absorbances of the standards against their respective concentrations and using the formula;

$Amount\ of\ MDA\ of\ sample\ /\ mg\ of\ protein$

Results were expressed as μmol MDA/mg protein

5.2.7.5 Antioxidant activity assays

Antioxidant activity was determined by using Ferric Reducing Antioxidant Power (FRAP) and Trolox Equivalent Antioxidant capacity (TEAC) assays. FRAP assay was carried out according to the method of Benzie and Strain (1996). FRAP reagent was prepared by mixing 30ml acetate buffer, 3ml TPTZ solution, 3ml $FeCl_3$ solution and 6.6ml distilled water. Aqueous solutions of 0, 25, 50, 100 and 200μM ascorbic acid were used as standards. 10μl of the cell homogenate/standards and 300μl of FRAP reagent was added to the well and incubated at 37°C

for 30 minutes. Absorbances were read at 593nm. Results are expresses as μmolAAE/mg protein. TEAC assay was done according to the method of Re *et al.* (1999) with some adaptations. ABTS radical cations were produced by reacting 88μl of the potassium-peroxodisulphate solution (7mM) and 5 ml of the ABTS solution (140mM). The mixture was left in the dark at room temperature for 24 hours and then diluted with ethanol in ratio 1:20 before use. 25μL of the sample/standards was added to 300μl of diluted ABTS and incubated for 30 minutes at room temperature. The absorbances were read at 734nm. A standard curve was prepared by using 0, 25, 50, 100, 200, 500μM solution of Trolox in ethanol.

5.2.8 Statistical analysis

All assays were done in triplicates and results data are presented as mean ± standard deviation. Statistical analysis was done by GraphPad prism 5 statistical package (GraphPad Software, USA), and significant differences among the samples were calculated using one-way ANOVA followed by Tukeys test at $p < 0.05$.

5.3 RESULTS

5.3.1 Cell proliferation and differentiation

Proliferation and differentiation of C2C12 cells was maintained with 10% and 2% fetal bovine serum (FBS) respectively. Differentiation of the cells was monitored and confirmed using an inverted microscopy and phase contrast (EVOS FL digital microscope). Fully differentiated C2C12 cells were characterized by a total change in morphology from single polygonal shaped cells (Figure 5.1a) into multinucleate long and densely packed myotubes (Figure 5.1b).

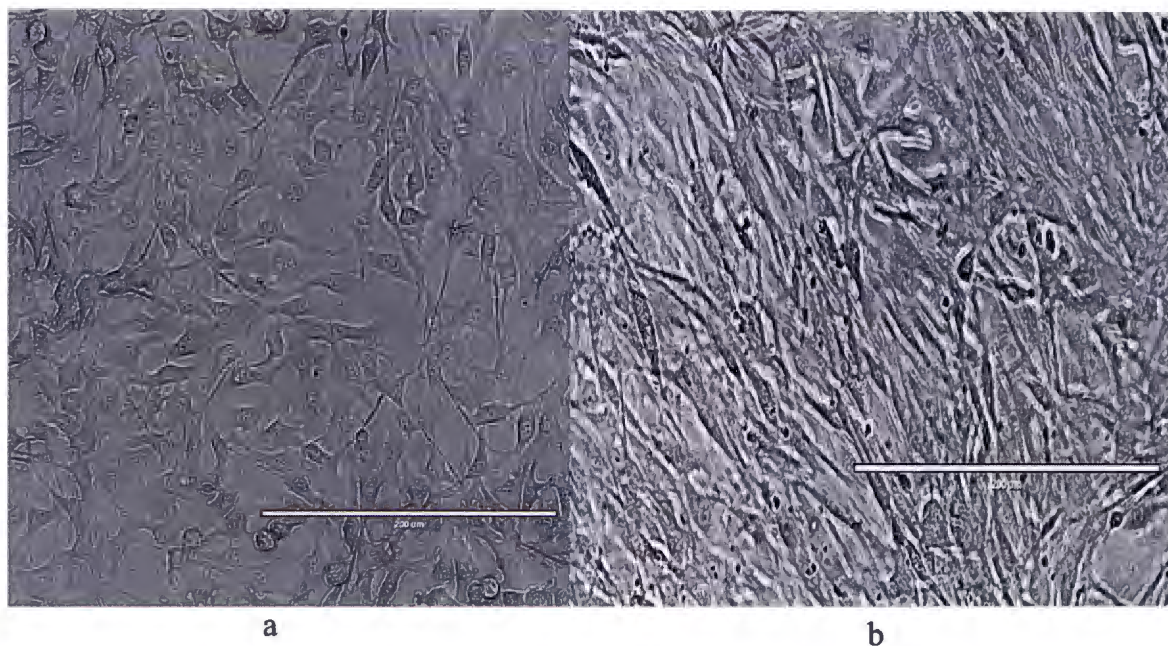


Figure 5.1 C2C12 cells (a) at 48 hours of culture prior to differentiation and (b) myocytes after 72 hours of differentiation

5.3.2 MTT cytotoxicity assay

Differentiated C2C12 cells were incubated with different concentrations (10-1000μg/ml) of OSPT and OSPL to determine the toxicity of the extracts. A percentage viability of more than 90% was recorded at concentrations up to 500μg/ml of OSPT and 100μg/ml of OSPL (Table 5.7). There were significant decreases in the cell viability of the cells above these concentrations. Therefore, 500μg/ml and 100μg/ml concentrations of OSPT and OSPL respectively were selected for this study.

Table 5.7: Percentage viability of C2C12 cells treated with different concentrations of OSPT and OSPL

	OSPT	OSPL
Concentration(μg/ml)	Cell Viability (%)	Cell Viability (%)
10	97.20 ± 1.56[a]	96.14 ± 0.08[a]
20	96.12 ± 1.04[a]	95.12 ± 1.72[a]
50	91.47 ± 2.16[a]	93.88 ± 0.09[a]
100	90.28 ± 2.12[a]	90.96 ± 2.34[a]
200	90.09 ± 1.87[a]	78.23± 1.11[b]
500	91.37 ± 1.68[a]	75.16 ±1.46[b]
1000	76.31 ± 2.01[b]	77.91 ± 0.65[b]

Values are mean ± SD (n=3). Bars with different letters denote statistical significant differences within the same column ($p < 0.05$). OSPT, Aqueous extract of tuber of orange fleshed sweet potato; OSPL, aqueous methanol extract of leaves of orange fleshed sweet potato

5.3.3 RNA integrity

RNA integrity was assessed using agarose gel electrophoresis. Lanes A, B, C, D, E, F, G and H as shown in figure 5.2 are the RNA bands of the 1kb Molecular weight marker, CONTROL, PT, PT+OSPT, PT+OSPL, PT+M, OSPT and OSPL respectively. All the samples had 18s size and 28s size bands confirming the intactness of extracted RNA.

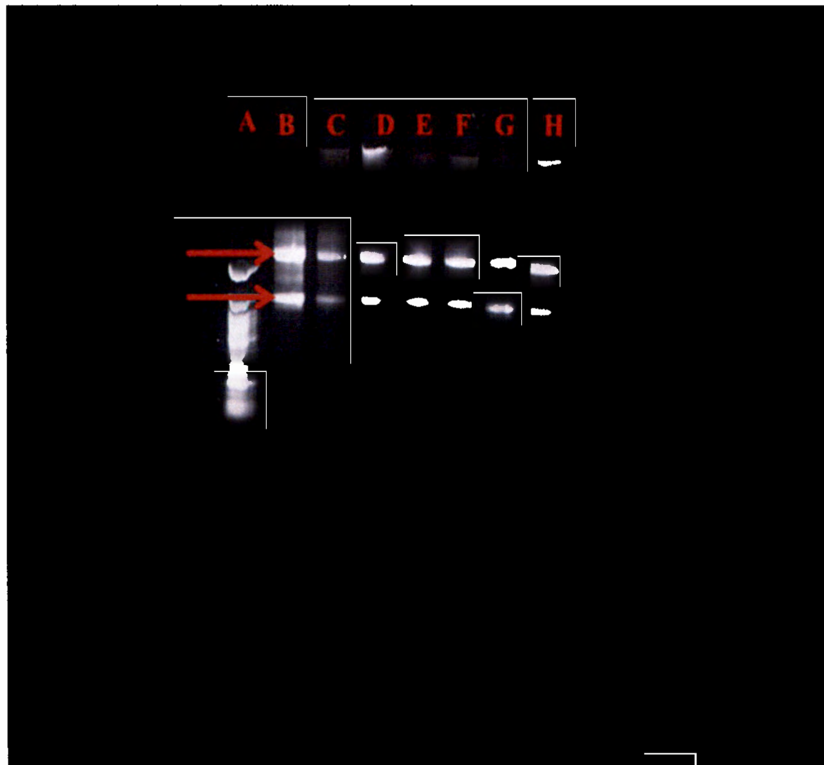


Figure 5.2: Agarose gel electrophoresis of extracted RNA samples from treated C2C12 myotubes. Lanes A, B, C, D, E, F, G and H are the RNA bands of the 1kb Molecular weight marker, CONTROL, PT, PT+OSPT, PT+OSPL, PT+M, OSPT and OSPL respectively.

5.3.4 Gene expression analysis in treated C2C12 myotubes

Gene expression was assessed using RT-qPCR technique and there were relative expression of all the diabetes related genes (GLUT4, NRF1, MEF2A, CPT1 and ACC2) and antioxidant enzymes genes (GPx and CAT) across all the experimental groups.

5.3.4.1 Effect of sweet potato extracts on expression levels of GLUT4, NRF1 and MEF2A genes

Results in figures 5.3(a,b and c) indicate the effect of orange fleshed sweet potato extracts on the relative expression level of GLUT4, NRF1 and MEF2A genes in treated differentiated C2C12 cells. After treatments, results show that there were significantly ($P<0.05$) lower expressions of GLUT4, NRF1 and MEF2A in the PT group when compared to the untreated CONTROL. However, there were significant increases in the genes' expression in the PT+OSPT and PT+OSPL groups when compared to the PT group, although the expressions were still significantly lower when compared to the untreated CONTROL and PT+M groups. The relative expressions of these genes were improved near normal in the PT+M group as they were not significantly different from the untreated CONTROL group. In the OSPT and OSPL groups, there was higher expression of all the genes although not significant ($P<0.05$) when compared to that of untreated control.

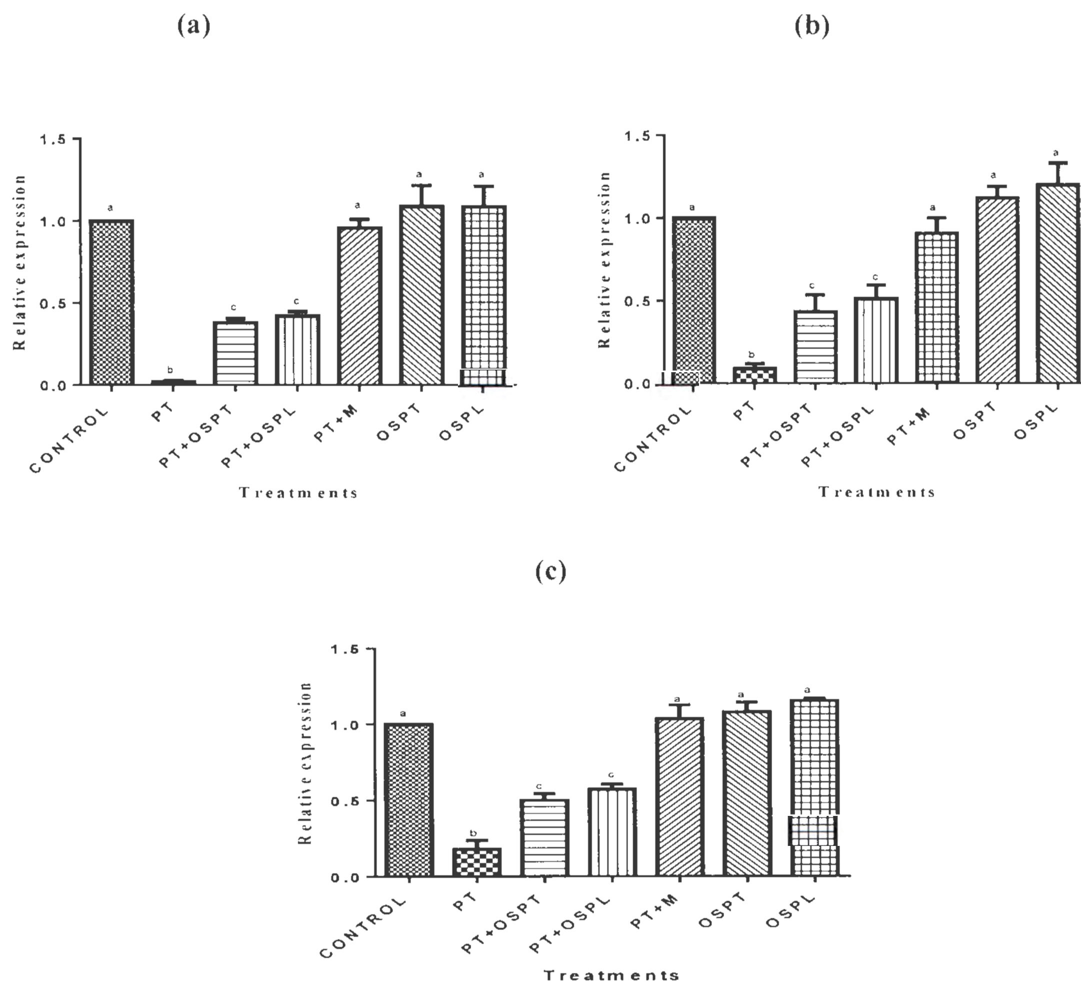


Figure 5.3: The effect of orange fleshed sweet potato extracts on the expression of (a) Glucose transporter 4 (GLUT4) (b) Nuclear respiratory factor 1 (NRF1) (c) Mycocyte enhance factor 2A (MEF2A) in treated C2C12 myotubes. Results are expressed as relative expression of mRNA with respect to untreated controls. Values are mean ± SD, (n=3). Bars with different letters denote statistical significant differences between results ($p < 0.05$). Treatment groups were palmitate (PT), palmitate and aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL).

5.3.4.2 Effect of sweet potato extracts on expression level of CPT1 and ACC2

The effect of orange fleshed sweet potato extracts on the relative expression level of CPT1 gene as shown in Figure 5.4 (a) indicates a significant decrease in expression in the PT group compared to the CONTROL group. On the other hand, in the PT+OSPT and PT+OSPL groups, there was a modest increase in the expression of CPT1, although not significantly different from the expression level in PT group. The relative expression of CPT1 in the PT+M group was not significantly different from the untreated CONTROL group. There was a higher expression of CPT1 in both the OSPT and OSPL groups when compared to that of untreated CONTROL group which was not significant at $P<0.05$. Furthermore, result in Figure 5.5 (b) shows a significant higher expression of the ACC2 gene in the PT group up to 4.2 fold of the CONTROL group at $p<0.05$. In the PT+OSPT and PT+OSPL groups, the extracts caused a significant reduction in the expression of ACC2 gene down to approximately 3 fold and 2 fold of the CONTROL respectively. The expressions in these 2 groups were still significantly different ($P<0.05$) from that of the CONTROL group. The expression of ACC2 gene decreased beyond the normal CONTROL although not significantly ($p>0.05$) in the PT+M, OSPT and OSPL groups.

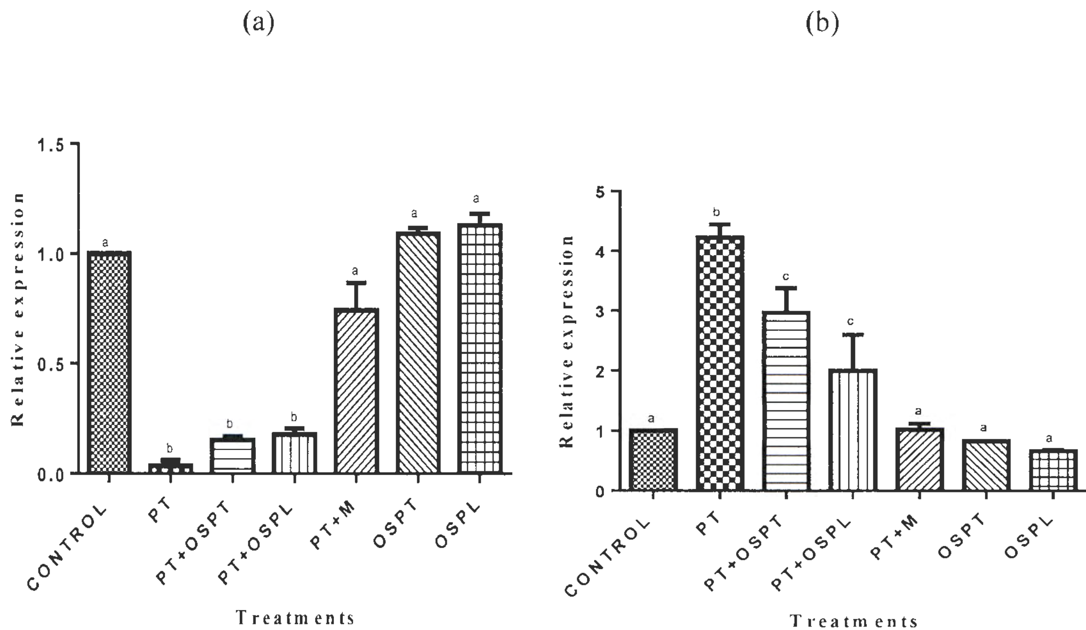


Figure 5.4: The effect of orange fleshed sweet potato extracts on the expression of (a) Carnitine palmitoyl transferase 1 (CPT1) and (b) Acetyl CoA carboxylase 2 (ACC2) in treated C2C12 myotubes. Results are expressed as relative expression of mRNA with respect to untreated controls. Values are mean ± SD, (n=3). Bars with different letters denote statistical significant differences between results ($p < 0.05$). Treatment groups were palmitate (PT), palmitate and aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL).

5.3.4.3 Effect of sweet potato extracts on expression level of antioxidant genes

Results in figure 5.5 indicate the relative expression levels of the antioxidant enzymes (GPx and CAT) in differentiated C2C12 cells across the experimental groups as measured by RT-qPCR technique. The expression levels of both GPx and CAT were significantly ($P<0.05$) lower in the PT group when compared to the untreated CONTROL and other groups. On the other hand, significant ($p<0.05$) increases in expression of both genes were recorded in the PT+OSPT and PT+OSPL groups when compared to the PT group. However, the expression levels in PT+OSPT and PT+OSPL groups were still significantly lower when compared to the untreated CONTROL group. In the PT+M group, the relative expressions of both genes were improved near normal as there were no significant differences when compared with the untreated CONTROL group. Moreover, for the CAT gene, there was no significant difference in the level of expression in the PT+M, PT+OSPT and PT+OSPL groups. In the OSPT and OSPL groups, there were higher expressions of both GPx and CAT genes although not significant when compared to that of untreated control.

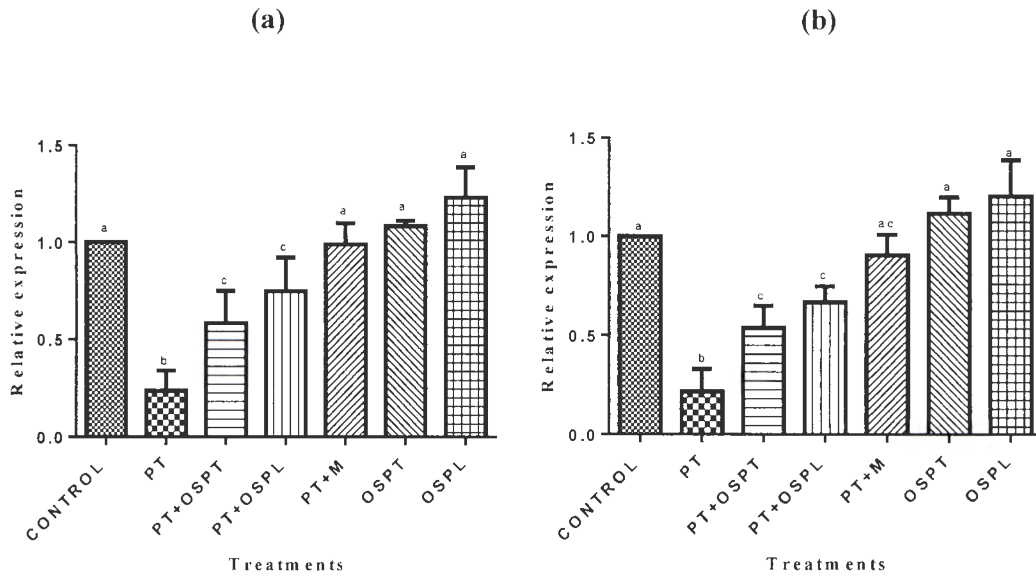


Figure 5.5: The effect of orange fleshed sweet potato extracts on the expression of antioxidant enzymes (a) Glutathione peroxidase (GPx) (b) Catalase (CAT) in treated C2C12 myotubes. Results are expressed as relative expression of mRNA with respect to untreated controls. Values are mean ± SD (n=3). Bars with different letters denote statistical significant differences between results ($p < 0.05$). Treatment groups were palmitate (PT), palmitate and aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL).

5.3.5 Effect of sweet potato extracts on oxidative stress parameters and antioxidant status

5.3.5.1 Effect of sweet potato extracts on total glutathione status and lipid peroxidation

The effect of the extracts of orange fleshed sweet potato on total glutathione status and lipid peroxidation across the groups are shown in figure 5.6 (a and b). Results show that there was a significantly ($P<0.05$) lower total glutathione (GSH) levels in the PT group when compared to the other groups. There were significant improvements in the level of expression in the PT+OSPT, PT+OSPL and PT+M groups when compared with the PT group. Furthermore, a significantly higher GSH level was observed in the OSPT and OSPL groups than in the untreated CONTROL group. On the other hand, there was a significantly higher level of malonaldehyde (MDA) in the PT than in the other groups at $P<0.05$. The levels of MDA in the PT+OSPT, PT+OSPL, PT+M, OSPT and OSPL groups were not significantly different from one another and that of the CONTROL group. In both the OSPT and OSPL groups, there were non-significant decreases in the levels of MDA when compared with the CONTROL group.

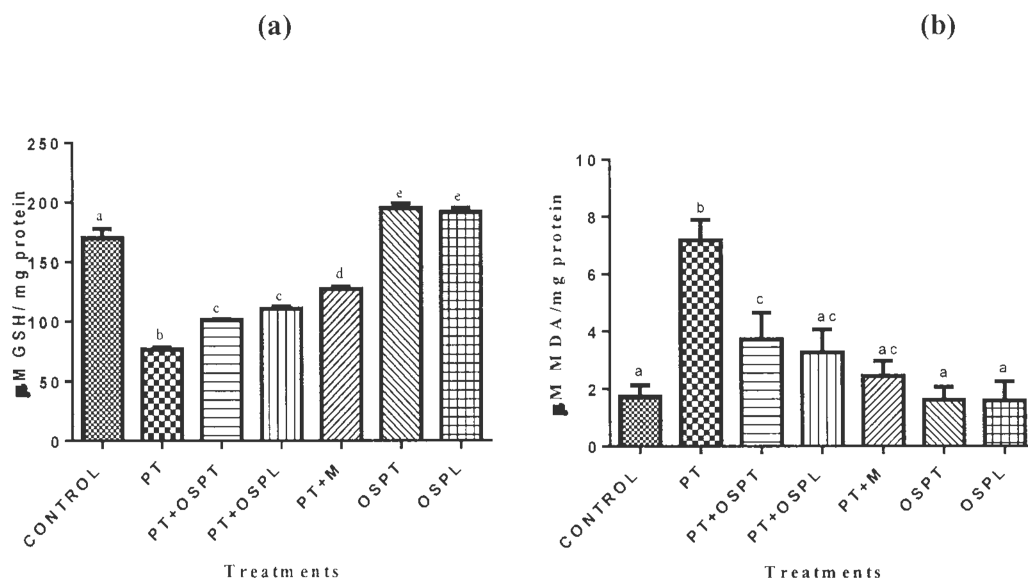


Figure 5.6: The effect of orange fleshed sweet potato extracts on (a) total glutathione status (GSH) and (b) lipid peroxidation in treated C2C12 myotubes. Values are mean ± SD (n=3). Bars with different letters denote statistical significant differences between results ($p < 0.05$). Treatment groups were palmitate (PT), palmitate and aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL).

5.3.5.2 Effect of sweet potato extracts on antioxidant enzymes activity

Results in figure 5.7 show the effect of the extracts of orange flesh sweet potato on glutathione peroxidase and catalase activities. The results indicate a significantly higher glutathione peroxidase (GPx) activity in the PT group than the other groups at $P<0.05$. The levels of GPx activity in the PT+OSPT, PT+OSPL, PT+M, OSPT and OSPL groups were not significantly different from one another and that of the CONTROL group. The level of catalase (CAT) activity was significantly lower in the PT group when compared with the CONTROL group. CAT activity improved in the PT+OSPT, PT+OSPL and PT+M groups although not significantly different from the PT group. In the OSPT and OSPL groups, there were non-significant increases in the activities of both antioxidant enzymes when compared with the CONTROL group.

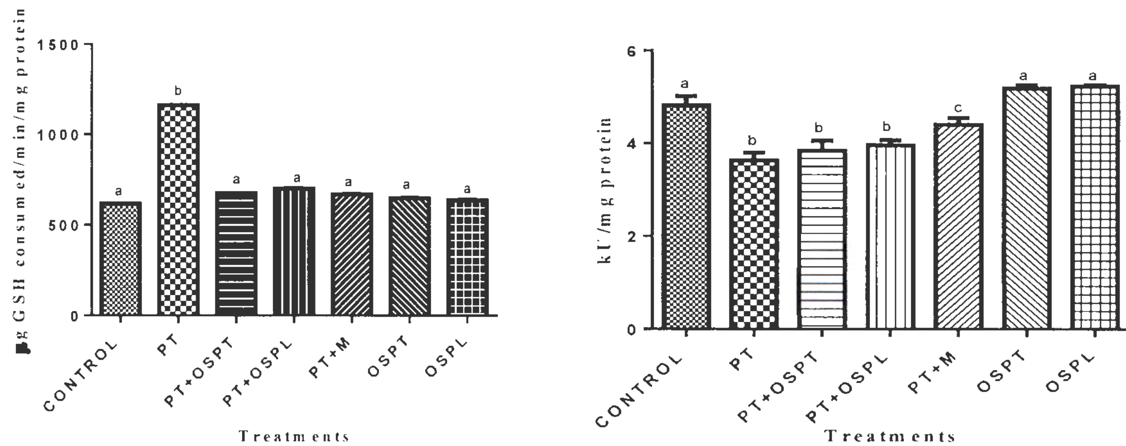


Figure 5.7: The effect of orange fleshed sweet potato extracts on (a) glutathione peroxidase (GPx) activity and (b) Catalase (CAT) activity in treated C2C12 myotubes. Values are mean ± SD (n=3). Bars with different letters denote statistical significant differences between results ($p < 0.05$). Treatment groups were palmitate (PT), palmitate and aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL).

5.3.5.3 Effect of sweet potato extracts on antioxidant capacity

The results of FRAP and TEAC values (Table 5.8) vary across the treatment groups in this study. Results obtained showed that there was a significant decrease in the FRAP and TEAC values in the PT group when compared with the CONTROL group. There was however, a significant improvement in the PT+OSPT and PT+OSPL and PT+M groups, although the values were still significantly lower than that of the control group. Furthermore, in the OSPT and OSPL groups, there is a significant increase in the FRAP and TEAC values when compared to the control group.

Table 5.8: The effect orange fleshed sweet potato extracts on antioxidant capacity (FRAP AND TEAC VALUES)

TREATMENT GROUPS	FRAP VALUES (μM AAE/mg protein)	TEAC VALUES (μM TE/mg protein)
CONTROL	271.0 ± 4.17^a	107.2 ± 1.68^a
PT	102.2 ± 5.06^b	39.8 ± 1.80^b
PT+OSPT	167.7 ± 0.54^c	71.94 ± 8.0^c
PT+OSPL	172.1 ± 1.94^c	76.19 ± 7.6^c
PT+M	251.3 ± 2.50^a	91.06 ± 1.40^a
OSPT	299.8 ± 2.5^d	127.9 ± 2.10^d
OSPL	296.9 ± 7.4^d	126.3 ± 2.51^d

Values are mean ± SD (n=3). Bars with different letters down a column denote statistical significant differences between results ($p < 0.05$). Palmitate (PT), palmitate and tubers' aqueous extract of orange fleshed sweet potato tubers (PT+OSPT), palmitate and leaves' aqueous-methanol extract of orange fleshed sweet potato leaves (PT+OSPL), palmitate and metformin (PT+M), aqueous extract of orange fleshed sweet potato tubers (OSPT), aqueous-methanol extract of orange fleshed sweet potato leaves (OSPL), metformin (M).

5.4 DISCUSSION

A large percentage of glucose disposal occurs in the skeletal muscle which is a major site involved in insulin-stimulated glucose uptake (Defronzo and Tripathy, 2009). Insulin resistance in skeletal muscle is characterized by ineffective glucose uptake and utilization in response to insulin leading to hyperglycemia (Abdul-Ghani and DeFronzo, 2010). There are studies that have reported the blood glucose lowering effect of sweet potato extracts (Ogunrinola *et al*., 2015; Pal *et al*., 2015). Aside the fact that these reports are mostly on the white fleshed cultivars, the molecular mechanisms behind the hypoglycemic effect of sweet potato has not been explored. In skeletal muscle, there are evidences of insulin resistance being attributable to free fatty acids (Turcotte and Fisher, 2008; Hiribara *et al*., 2010; Martins *et al*., 2012). Palmitate is a saturated fatty acid that has been used in different *in vitro* studies to create insulin resistant models (Park *et al*., 2009: Mazibuko *et al*., 2013).

In this study, insulin resistance in differentiated C2C12 cells was induced by incubating the cells in 0.75mM palmitate followed by treatment with 500µg/ml and 100µg/ml of OSPT and OSPL respectively. The expression levels of the genes (GLUT4, NRF1, MEF2A, CPT1, ACC2, GPx and CAT) were determined relative to a housekeeping gene (GAPDH) and as a fold change in expression of the control group. The results showed that there were significant decreases in expression of GLUT4, NRF1 and MEF2A in the palmitate treated group when compared with the control group. GLUT4 is an insulin-sensitive major transporter of glucose in the skeletal muscle while NRF1 and MEF2A are important co-transcription factors that are involved in the regulation of its expression. The expression and activity of GLUT4 have been found to be directly correlated with that of NRF1 and MEF2A as well as with insulin sensitivity and the maintenance of glucose homeostasis in skeletal muscle. Insulin resistance is associated with a

reduced expression of GLUT4 gene and its protein (Doehner *et al*., 2010; Kampmann *et al*., 2011). The reduced expressions of these genes confirm the insulin resistant conditions of the cells. The overexpression of NRF1 in transgenic muscle resulted into increase in expression of MEF2A and GLUT4 which consequently enhance glucose uptake (Baar *et al*., 2003). Gumede and Ojuka, 2011 reported that NRF1 overexpression leads to increase in GLUT4 expression through MEF2A. Therefore, upregulating the expression of GLUT4 and its transcription factors is an important therapeutic strategy in the management of insulin resistance. The results of this study showed upregulated expression of GLUT4, NRF1 and MEF2A in the insulin resistant cells upon treatment with OSPT and OSPL. Furthermore, there was a significant decrease in expression of CPT1 and increase in expression of ACC2 in the palmitate treated C2C12 cells when compared to the untreated control group. However, an increase in the expression of CPT1, although not significantly, and a significant decrease in the expression of ACC2 (3 and 2 folds) were observed upon treatments with OSPT and OSPL respectively. Accumulation of intracellular lipids has been implicated in the development of insulin resistance in skeletal muscle (Silveira *et al*., 2008; Hiribara *et al*., 2010; Martins *et al*., 2012). ACC2 and CPT1 are important regulators of mitochondrial fatty acid oxidation and hence strategies that influence their expression would affect the level of intracellular lipids and hence have therapeutic implications in the management of insulin resistance in skeletal muscle. Choi *et al*., (2007) reported increased fatty acid oxidation, reduced diacylglycerol content and improved insulin sensitivity in ACC2 knockout mice. This is consistent with earlier findings of Rosa *et al*. (2003) which attributes the reversal of insulin sensitivity to the downregulation of ACC2 gene in the skeletal muscle of previously obese subjects after undergoing bariatric operation. Overexpression of CPT1 in skeletal muscle resulted into improved insulin sensitivity in high-fat diet induced insulin resistance (Sebastian *et*

al., 2007; Bruce *et al.*, 2009). These evidences suggest that increased and decreased expression of CPT1 and ACC2 respectively observed in PT+OSPT and PT+OSPL groups indicate that aqueous methanol extracts of orange fleshed sweet potato have the potential to improve insulin sensitivity in insulin resistant skeletal muscle.

Oxidative stress is believed to be involved in the impairment of insulin signaling and the development of insulin resistance (Rains and Jain, 2011; Balbaa *et al.*, 2017). Oxidative stress has been associated with insulin resistant status in skeletal muscle (Evans *et al.*, 2005; Houstis *et al.*, 2006). Ameliorating oxidative stress is therefore one of the therapeutic strategies aimed at improving insulin sensitivity. Glutathione peroxidase (GPx) and catalase (CAT) are important antioxidant enzymes responsible for the scavenging of ROS and hence are considered essential determinants of antioxidant status in the mammalian system (Yang and Lee, 2015). There have been conflicting reports on the effect of oxidative stress on the expression of the different antioxidant enzymes. Some studies have reported decrease, while others have reported increase or even unchanged expression of GPx and CAT in insulin resistant or diabetic conditions (El-Bahr, 2013). In this study, there was a significant reduction in the expression of both GPx and CAT genes in the palmitate-induced insulin resistant groups. The expression of both antioxidant genes increase significantly upon treatment with OSPT and OSPL and metformin. For all the genes except ACC2, and although not significantly, the level of expression was higher in OSPL and PT+OSPL groups than in the OSPT and PT+OSPT groups respectively. This can possibly be due to the fact that OSPL which is the extract from the leaves of the orange fleshed sweet potato contains more polyphenol compounds than OSPT, the extract from the tubers as demonstrated in a prior experiment in this study.

Glutathione (GSH) is an endogenously synthesized antioxidant and its depletion is one of the indices of oxidative stress in living cells (Mytilineou *et al.*, 2002; Kerksick and Willoughby, 2005). In this study, the total glutathione status was lower in the palmitate-treated group and significantly improved upon treatment with OSPT, OSPL and metformin. This demonstrated that OSPT, OSPL and metformin were able to ameliorate the oxidative stress induced by the palmitate treatment. The activity of antioxidant enzymes are considered indicative of the antioxidant status of a biological system (Yang and Lee, 2015). Glutathione peroxidase (GPx) is an antioxidant enzyme which catalyzes the reduction of hydrogen peroxide and lipid peroxides into water and lipid alcohols respectively using glutathione as reductant (Tappel, 2014). GPx activity was significantly higher in the palmitate-treated groups compared to all other groups. A possible reason for increased GPx activity during oxidative stress is the increased ROS production, which means GPx would work more to scavenge ROS while using and depleting the intracellular glutathione level (Torres *et al.*, 2011). Another important antioxidant enzyme which protects cells from oxidative damage is catalase. Catalase, which has one of the highest turnover rates of all enzymes, catalyzes the decomposition of hydrogen peroxide into water and oxygen (Kabel, 2014). In the palmitate-treated groups, there was a significant decrease in CAT activity when compared to the control group. However, the activity increased, although not significantly, following treatments with OSPT, OSPL and metformin. Lipid peroxidation is the oxidative degradation of lipids caused by reactive oxygen species resulting in cellular damage (Nikki *et al.*, 2005). Malonaldehyde (MDA) is a product of lipid peroxidation which is a useful biomarker of oxidative stress in cells (Yoshida *et al.*, 2013). The level of MDA was measured across the treatment groups and results showed a significantly higher MDA in the palmitate treated group than all the other groups. There was a significant reduction of MDA upon

treatments with the extracts and metformin. FRAP and TEAC values are both measures of antioxidant capacity relative to a standard antioxidant compound. FRAP and TEAC values of the palmitate treated groups were significantly lower and there were significant improvements of both values following treatment with the extracts and metformin.

5.5 CONCLUSION

Generally, across all the experiments, treatment with the extracts of both the leaves and tubers of orange fleshed sweet potato were not as effective as the standard metformin treatment. However, this study has demonstrated the ability of the extracts to influence the expression of type 2 diabetes related and antioxidant genes as well as ameliorate oxidative stress in insulin-resistant C2C12 cells. These findings demonstrate the potential of orange fleshed sweet potato as a phytotherapy agent against insulin resistance and type 2 diabetes.

CHAPTER SIX

EFFECTS OF EXTRACTS OF ORANGE FLESHED SWEET POTATO CULTIVAR ON CARBOHYDRATE METABOLISING ENZYMES

ABSTRACT

The inhibition of carbohydrate metabolizing enzymes is an important strategy adopted in the management of type 2 diabetes to achieve reduction of postprandial hyperglycemia. Edible plants rich in phenolic compounds have been reported to act as good inhibitors of carbohydrate digestive enzymes. This study assessed the inhibitory potential of the extracts of tubers (OSPT) at (1, 2, 3, 4 and 5) mg/ml and leaves (OSPL) at (10, 20, 30, 40 and 50)mg/ml of orange fleshed sweet potato on α-glucosidase, α- amylase, sucrase and maltase using acarbose (0.625, 1.25, 2.5, 5, 10) mg/ml as standard. Both extracts show significantly lesser inhibition and higher IC_{50} values of the activities of all the enzymes compared to acarbose at ($P<0.05$). OSPT had the highest IC_{50} values of (35.03 ± 1.86, 38.38 ± 2.34, 51.54 ± 2.76 and 70.93 ± 1.98) mg/ml for α-glucosidase, α-amylase, sucrase and maltase respectively. However, OSPL showed an appreciable inhibition of all the enzymes and a significantly lower IC_{50} of (5.31 ± 0.92, 5.4 ± 1.82, 4.14 ± 2.86, and 5.46 ± 3.01) mg/ml for alpha glucosidase, alpha amylase, sucrase and maltase respectively at ($P<0.05$). The findings from this study indicate the potential of the leaves of orange fleshed sweet potato in the management of hyperglycemia through its inhibitory effects on carbohydrate metabolizing enzymes.

6.1 INTRODUCTION

Type 2 diabetes is a metabolic disorder characterized by hyperglycemia caused by insulin resistance and/or insulin deficiency (Lebovitz, 1999). One of the strategies that have been employed in the management of type 2 diabetes is the inhibition of carbohydrate metabolizing enzymes that are responsible for the breakdown and digestion of dietary carbohydrates into glucose (Chakrabarti and Rajagopalan, 2002). This therapeutic approach is adopted to control postprandial hyperglycemia, which is a hallmark in type 2 diabetes and its complications. Alpha-glucosidase, alpha-amylase, sucrase and maltase are important enzymes involved in the digestion of carbohydrates into glucose (Nair *et al.*, 2013). There are synthetic drugs such as acarbose which are inhibitors of alpha-glucosidase or alpha-amylase, but the use of these synthetic drugs has been associated with gastrointestinal side effects (Kim *et al.*, 2005). There is, therefore, an increasing interest in the search for potent inhibitors of carbohydrate metabolizing enzymes from plants and its products (Kumar *et al.*, 2011; Mogale *et al.*, 2011; Kazeem *et al.*, 2013; Kazeem *et al.*, 2015; Ramprasad *et al.*, 2016). Sweet potato is a food crop with different varieties or cultivars being cultivated across the world. Generally, hypoglycemic effects of sweet potato and its extracts have been reported in both *in vivo* and *in vitro* studies (Surayia *et al.*, 2008; Ogunrinola *et al.*, 2015) However, the ability of sweet potato extracts to inhibit carbohydrate digestive enzymes have not been explored to determine if this is one of the mechanisms through which it exerts its hypoglycemic effects. Orange fleshed sweet potato cultivars are particularly rich in carotenoids, polyphenolic compounds and ascorbic acids which are very potent antioxidants (Alam *et al.*, 2016; Rautenbach *et al.*, 2010). Amelioration of oxidative stress by antioxidants is another strategy that is employed in the management of type 2 diabetes and its complications. Antioxidant effects and ability to inhibit carbohydrate metabolizing enzymes are

desirable combined attributes in the quest for an ideal medicinal plant in the management of type 2 diabetes (Mai *et al*., 2007; Kaskoos, 2013; Dewi and Maryani, 2015). This study investigated *in vitro,* the ability of the extracts of orange-fleshed sweet potato cultivar to inhibit alpha-glucosidase, alpha amylase, sucrase and maltase enzymes.

6.2 MATERIALS AND METHOD

6.2.1 Plant samples

Orange fleshed sweet potato was obtained from the Department of crop science, North-West University, Mafikeng campus, South Africa. The leaves and root tubers were collected fresh from the university farm. They were rinsed with running water. Leaves were cut into pieces and tubers into thin chips before being air dried in the laboratory at room temperature (25 ± 2°C). Air dried samples were ground into fine powder (about 500μm mesh size) and packed in an air tight container. They were labeled accordingly and stored at 4°C for further use.

6.2.2 Preparation of extracts

Preparation of crude aqueous and aqueous-methanol (l:1) extracts of the tubers' and leaves' extracts respectively was done by soaking 5g of the powder in 100ml of the respective solvents for 24 hours at room temperature with constant shaking. Extracts were recovered through lyophilization and evaporation using a freeze dryer (Alpha 1-4 LSC Plus) and a rotary evaporator (RE-52A) respectively. Dried aqueous extracts of the tubers (OSPT) and aqueous-methanol extract of the leaves (OSPT) were stored in the dark at -20°C.

6.2.3 α-glucosidase, sucrase and maltase inhibition assay

The effect of the extracts on α-glucosidase activity was determined by the method of Adisakwattana *et al*. (2009) using α-glucosidase enzyme from rat intestinal acetone powder. The

substrates; 5mM p-nitrophenyl glucopyranoside (pNPG), 25 mM maltose and 50mM sucrose were prepared in 0.1M sodium phosphate buffer. Rat intestinal acetone powder was homogenized in 0.9% NaCl (0.3mg/ml). The mixture was centrifuged at 12,000g for 30 minutes and 100μl of the crude enzymes was pre-incubated with different concentration of the leave (1, 2, 3, 4 and 5) mg/ml and tuber (10, 20, 30, 40 and 50) mg/ml extracts for 10 minutes. Fifty microliters (50μl) of the substrates were added to initiate the reaction while the mixture was incubated at 37°C for 30 minutes. The reaction was terminated by adding 2 ml of 0.1 M Na_2CO_3 and the enzymes activities were determined by measuring the absorbance of the resulting products at 405 nm (apha-glucosidase) and 540 nm (maltase and sucrose). The results were expressed as percentage of the blank control. Percentage inhibition was calculated as

$\%$ inhibition= $[(A_0 - A_S)/A_0] \times 100$.

A_0 and A_S are the absorbances of the control and extracts respectively.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

6.2.4 α-Amylase inhibition assay

The effect of the extracts on the activity of α-amylase was determined according to the modified method of McCue and Shetty (2004). Alpha-amylase solution (0.5mg/ml) was prepared in 0.02 M sodium phosphate buffer (pH 6.9). Three hundred microliters (300μl) of the leaves (1, 2, 3, 4 and 5) mg/ml and tuber (10, 20, 30, 40 and 50) mg/ml extracts was added to 300 μl of α-Amylase solution in Eppendorf tubes. The reaction mixture was incubated at 25°C for 10 minutes, after which 300 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added. The solution was then incubated again at 25°C for 10 minutes. The reaction was

stopped by the addition of 600µl of dinitrosalicylic acid (DNS) reagent. The tubes were incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted with 5 ml distilled water and absorbance was measured at 540 nm using a spectrophotometer. Distilled water was used as control and acarbose as standard. The α-amylase inhibitory activity was calculated as % inhibition = $[(A_0 - A_S)/A_0] \times 100$.

A_0 and A_S are the absorbances of the control and extracts respectively.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

6.2.5 Statistical analysis

Statistical analysis was done using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Turkey's test. All the results data were expressed as mean ± standard deviation.

6.3 RESULTS AND DISCUSSION

Inhibition of carbohydrates digestive enzymes is an important strategy in the management of type 2 diabetes. This study investigated the inhibitory activities of the extracts of both tubers and leaves of orange-fleshed sweet potato against alpha-amylase, alpha-glucosidase, sucrase and maltase using acarbose as standard. The concentrations of sample to inhibit 50% of enzyme activity were calculated as IC_{50}. IC_{50} is a standard measure of the efficacy and performance of a therapeutic agent or candidate. It is the concentration of a drug that results into 50% inhibition of a particular biological process (Sebaugh, 2011).

Figures 6.1-6.4 show the percentage inhibitions of alpha-glucosidase, alpha amylase, sucrase and maltase respectively by OSPT, OSPL and acarbose. Both extracts showed significantly weaker inhibition of all the enzymes compared to the standard acarbose ($P<0.05$). Nevertheless, OSPL showed a significantly stronger inhibition than OSPT.

Table 6.1 shows the IC_{50} values of OSPT, OSPL and acarbose for all the enzymes. Acarbose showed significantly lower IC50 values of 0.194 mg/ml, 0.11mg/ml and 0.04mg/ml for alpha-glucosidase, sucrase and maltase activities respectively when compared with both extracts. The IC_{50} values for alpha glucosidase, sucrase and maltase in OSPT are 35.03mg/ml, 51.54mg/ml and 70.93mg/ml, while that of OSPL are 5.31mg/ml, 4.14mg/ml and 5.46mg/ml respectively. There are significantly lower IC_{50} values of alpha glucosidase, sucrase and maltase activities in OSPL than in OSPT at ($P<0.05$).

Similarly, acarbose also showed more potent inhibition of the alpha amylase than the extracts. The IC50 values are 0.47mg/ml, 38.38mg/ml and 5.40mg/ml for acarbose, OSPT and OSPL respectively. OSPL showed a significantly lower IC_{50} value than OSPT at ($P<0.05$). The extracts

of orange fleshed sweet potato showed a considerable inhibition of the carbohydrate metabolizing enzymes with the leaves' extract being a better inhibitor of the enzymes than the tubers' extract. The inhibitions of alpha-glucosidase, alpha amylase, sucrase and maltase by plants have been linked to the presence of phenolic compounds (Mai *et al.*, 2007; Miao *et al.*, 2016). Guava (Deguchi *et al.*, 1998; Mai *et al.*, 2007) and green teas (Hara and Honda, 1992; Mai *et al.*, 2007) are some of the edible plants that have shown inhibitory activities against digestive enzymes. The rate of inhibition of these enzymes has also been found to be directly correlated with the concentration of phenolic compounds present in these plants (Mai *et al.*, 2007). The stronger inhibition of the enzymes' activities in the leaves' extract could be due to the presence of higher concentration of phenolic compounds in the leaves than in the tubers. A prior study (not yet published) which analyze the phytochemicals present in the leaves and the tubers of the orange fleshed sweet potato cultivar used in this study showed a significantly higher content of phenolic compound in the leaves than in the tubers. This is also consistent with the works of Padda and picha, (2008). Orange- fleshed sweet potato cultivars are rich in flavonoids and phenolic acid which have been reported as some of the phytochemicals responsible for the inhibitory actions of medicinal plants on carbohydrate metabolizing enzymes (Tadera *et al.*, 2006; Kwon *et al.*, 2008). Generally, the results from this study indicate that extract of orange-fleshed sweet potato is a milder inhibitor of the carbohydrate metabolizing enzymes in comparison with acarbose. This could be a desirable outcome as a lesser inhibition of the enzymes would probably result in lesser side effects associated with synthetic drugs like acarbose.

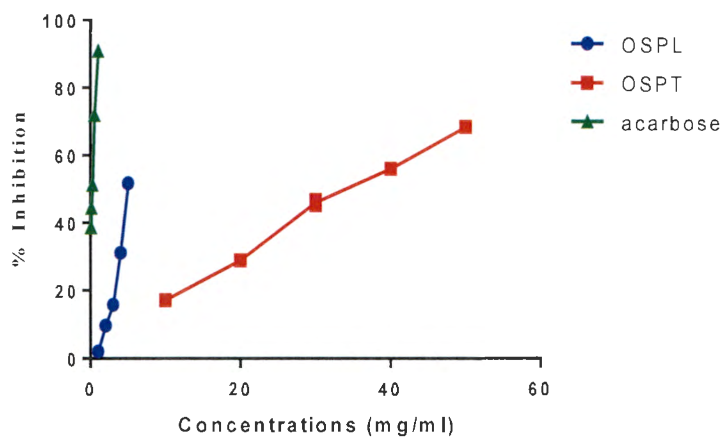


Figure 6.1: Percentage inhibition of orange fleshed sweet potato extracts and acarbose on α-glucosidase. OSPT, aqueous extract of orange fleshed sweet potato tubers; OSPL, aqueous-methanol extract of orange fleshed sweet potato leaves.

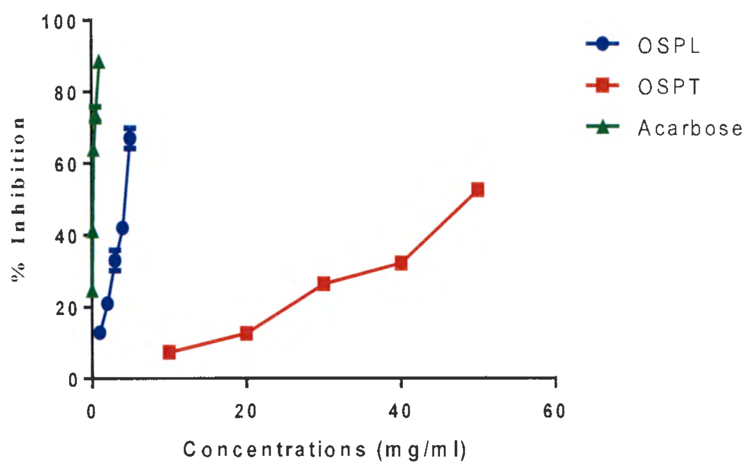


Figure 6.2: Percentage inhibition of orange fleshed sweet potato extracts and acarbose on sucrase. OSPT, aqueous extract of orange fleshed sweet potato tubers; OSPL, aqueous-methanol extract of orange fleshed sweet potato leaves.

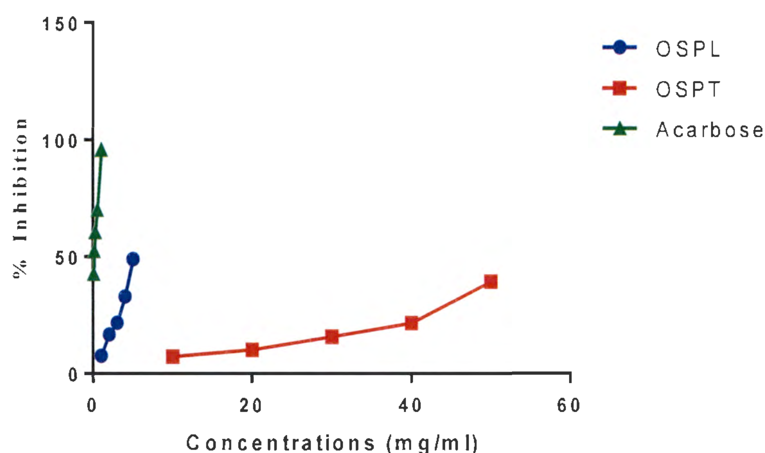


Figure 6.3: Percentage inhibition of orange fleshed sweet potato extracts and acarbose on Maltase. OSPT, aqueous extract of orange fleshed sweet potato tubers; OSPL, aqueous-methanol extract of orange fleshed sweet potato leaves..

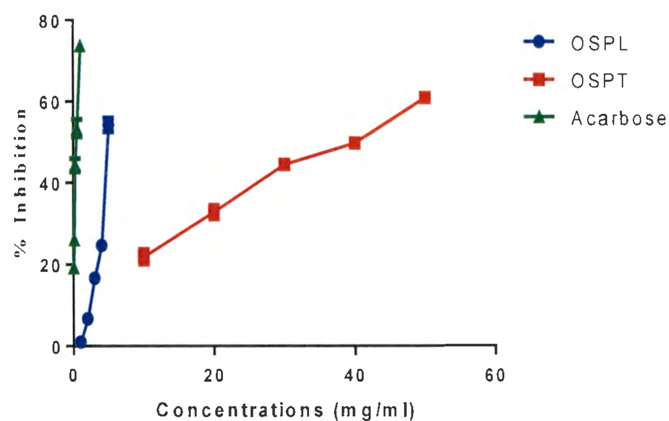


Figure 6.4: Percentage inhibition of orange fleshed sweet potato extracts and acarbose on α-amylase. OSPT, aqueous extract of orange fleshed sweet potato tubers; OSPL, aqueous-methanol extract of orange fleshed sweet potato leaves.

Table 6.1: IC_{50} values for α-glucosidase, sucrose, maltase and α –amylase inhibitory potential of the extracts of orange fleshed sweet potato and acarbose.

	IC_{50} (mg/ml)			
	α-glucosidase	Sucrase	Maltase	α-amylase
OSPT	35.03 ± 1.86[a]	51.54 ± 2.76[a]	70.93 ± 1.98[a]	38.38 ± 2.34[a]
OSPL	5.31 ± 0.92[b]	4.14 ± 2.86[b]	5.46 ± 3.01[b]	5.4 ± 1.82[b]
Acarbose	0.194 ± 0.45[c]	0.11 ± 0.87[c]	0.04 ± 1.56[c]	0.47 ± 1.34[c]

The values are expressed as means ± SD of triplicate tests, (n=3). Means down vertical column with different letters are significantly different (*P* <0.05). OSPT, aqueous extract of orange fleshed sweet potato tubers; OSPL, aqueous-methanol extract of orange fleshed sweet potato leaves.

6.4 CONCLUSION

The results of this study indicate that the leaf extracts of orange fleshed sweet potato were able to appreciably inhibit *in vitro* the activities of carbohydrate metabolizing enzymes. This activity may be attributed to the high content of polyphenolic compounds present in the leaves of orange fleshed sweet potato. Isolation and characterization of compounds that may be responsible for this inhibition would be an important step in the exploration of the anti-diabetic potential of sweet potato.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMM ENDATION

7.1 GENERAL DISCUSSION AND CONCLUSION

Sweet potato is a food crop being cultivated across the world. It is an important root crop in South Africa, especially in the northern subtropical regions of the country (Laurie *et al.*, 2017). Food security and nutrition is important in the fight against malnutrition particularly in developing countries. Sweet potato is a traditional crop that has the potential to provide affordable and alternate source of food nutrients (Padmaja *et al.*, 2012). Crop improvement informed by different breeding goals has led to the development of different cultivars of sweet potato in different parts of the world including South Africa. As seen in other plants, significant variations in chemical composition usually exist among the different cultivars of sweet potato (Harrison *et al.*, 2003; Ji *et al.*, 2015). Therefore, knowledge of the nutritional qualities of existing cultivars of this food crop is important for its promotion as an alternative source of food.

As expected, all the nutritional components measured in this study vary across the different cultivars. The difference can mostly be attributed to genetic differences as all the cultivars were cultivated and grown under the same condition and in the same geographical area. Overall, the protein and fibre contents of the cultivars were higher than the previously reported values for cultivars in Rwanda (Ingabire and Hilda, 2011), Benin republic (Sanoussi *et al.*, 2016), Nigeria (Omodamiro *et al.*, 2009), Bangladesh (Alam *et al.*, 2016) and Brazil (Rodriques *et al.*, 2016). Dietary proteins and fiber are essential for proper functioning and development of the body (Alayande *et al.*, 2012). High dietary fiber content decreases blood cholesterol, enhances digestibility and reduces inter-colonic pressure thus also lessen the risk of the development of

large bowel cancers (Al-Farga *et al*., 2016). Food rich in fibre are considered beneficial for type 2 diabetic patients especially towards the improvement of glycemic control (Dworatzek *et al*., 2013). Generally, the total fatty acid contents of the cultivars are lower than the other nutritional components. They are comparable with previously reported values for cultivars grown in Brazil (Rodriques *et al*., 2016) and lower than previously reported for cultivars in Nigeria (Omodamiro *et al*., 2013; Anthony *et al*., 2014). However, the fatty acid contents are higher than those reported for cultivars in Bangladesh (Alam, 2016). High fiber and low fat food consumption combined with regular exercise have been shown to be able to reduce the risk of breast cancer in postmenopausal women (Barnand *et al*., 2006). The ash contents of the cultivars demonstrated that they are relatively good sources of minerals as they are higher than those previously reported in literature. The cultivars also have considerable carbohydrates, total sugar and energy levels when compared with previous reports. Propanoic acid, benzoic acid, cinnamic acid and malic acid which are some of the phytochemicals that were detected in the cultivars have been reported to have medicinal values such as hepatoprotection, antioxidant, anticancer and antiviral properties (Niero *et a*l., 2013; Suprayitno, 2015 ; Elmezian *et al*., 2016).

Antioxidant profiles of plants are considered important index of their therapeutic potential (Maksimovic *et al*., 2005). The therapeutic effects of most medicinal plants have been attributed to their antioxidant properties (Sabu and Kuttan, 2002; Makhafola *et al*., 2016; Miled *et al*., 2017). Polyphenols are a major group of phytochemical that contributes largely to the antioxidant properties of plants (Kerio *et al*., 2013; Pajak *et al*., 2014). The total flavonol, flavanol, flavonoid and polyphenol contents vary significantly across the different cultivars. Cultivar variation of phytochemical profiles in different plants including sweet potato has been reported in various plants such as apple (Xu *et. al*., 2016) wheat (Adom *et. al*., 2003; Jeong *et.*

al., 2017), grapes (Katalinic *et. al.*, 2009) and even in sweet potato (Montilla *et. al.*, 2011; Park *et. al.*, 2016). In this study, the orange fleshed sweet potato had higher level of antioxidant profiles than the white fleshed cultivars which is consistent with the findings of the studies of Tang *et al.* (2015) and Park *et al.* (2016). Furthermore, leaves of sweet potato have been reported to accumulate more polyphenols than the tubers (Islam *et al.*, 2002; Padda and Picha, 2007). This was also demonstrated in this study as the leaves of the sweet potato cultivars contained up to 30 folds of the antioxidant profiles found in the tubers. Generally in the leaves' extracts, the aqueous-methanol extracts of the sweet potato cultivars had better antioxidant profiles than the aqueous extracts. This suggests that the polyphenolic compounds in the leaves are more soluble in aqueous methanol extract than in pure aqueous solvent. On the contrary, the aqueous extracts of the tubers mostly had more antioxidant content and stronger antioxidant activity than the aqueous- methanol extracts. Solvents and plant parts have been reported as important factors that affect the level of phenolics and antioxidant activity in plant extracts (Amensour, 2010). The antioxidant activity of the cultivars were determined by the FRAP, TEAC and NO scavenging activity assays. Antioxidant activities of plants are through diverse mechanisms such as decomposition of hydrogen peroxides, reductive capacity and radical scavenging ability (Amoateng *et al.*, 2011). Thus, antioxidant activity of a sample is best determined when more than one antioxidant test method is employed (Alam *et al.*, 2013). The FRAP and TEAC values of the extracts are indicative of their antioxidant strength. IC_{50} values of NO scavenging activity of the extracts were estimated from the percentage inhibitions at different concentrations of the extracts. The extracts demonstrated the ability to scavenge nitric oxide at varying levels. The aqueous-methanol extract of 'Bophelo' exhibited the strongest antioxidant activity with the FRAP and TEAC methods in both the tubers and leaves of the cultivars. It also showed the

strongest NO radical scavenging activity in the leaves while aqueous extract of '199062.1' showed the highest activity in the tubers. There is a significant and strong correlation between the total polyphenol contents of the cultivars and the antioxidant activity as determined by FRAP and TEAC methods. This means that phenolic compounds are mostly responsible for the antioxidant activity displayed by the extracts. However, the correlation between the total polyphenols and NO radical scavenging was a moderate one which suggests there are possibly other radical scavenging compounds apart from phenolics in the extracts. The antioxidant ability of phenolic compounds has been attributed to the redox potential of their phenolic hydroxyl groups which enables them to act as reducing agents (Amoateng *et al.*, 2011).

Individual chromatograms of the LC-MS analysis (addendum 2) of the extracts of the different cultivars showed the presence of some polyphenolic compounds at varying concentrations. Hyperoside, caffeic acid, rutin and qurcetin were detected in the extracts of all the cultivars at varying level. These compounds are polyphenols with strong antioxidant capability (Pulido *et al.*, 2000). Given that there is a strong correlation between total polyphenol contents and antioxidant activity, these compounds, at least, contribute to the antioxidant activity displayed by the extracts.

Due to increasing evidence of the involvement of oxidative stress in insulin resistance, antioxidant therapy is an emerging strategy in the management of insulin resistance in type 2 diabetes. Dietary antioxidants are mostly already part of human daily diet and are now being explored for their antioxidative properties and therapeutic potential as they offer cheap alternative therapy in the management of type 2 diabetes. The antidiabetic potential of sweet potato, especially the white fleshed cultivars has been reported by a number of studies (Kusano and Abe, 2000; Ludvik *et al.*, 2002). There has been little or no report of the antidiabetic

NWU LIBRARY

potential of orange-fleshed sweet potato even though they also contain antioxidant compounds. Therefore, Bophelo, an orange fleshed sweet potato which was found to exhibit a strong antioxidant activity in an earlier phase of this study was explored for its antidiabetic potential in insulin resistant C2C12 cells. The aqueous methanol extracts of the tubers and leaves were able to ameliorate oxidative stress and improve antioxidant status in palmitate treated C2C12 cells. Palmitate is a saturated fatty acid capable of inducing oxidative stress mediated-insulin resistance (Park *et al.*, 2015). The results of this study demonstrated a significant reduction and increase in the total glutathione status (GSH) and level of malonaldehyde (MDA) respectively in the palmitate treated C2C12 cells. The reduction in GSH level and increase in the level of MDA indicated a condition of oxidative stress with the cells. Upon treatment with 100µg and 500µg of the leaves and tubers' extracts respectively, the GSH level increased and the level of MDA decreased significantly. These findings suggest that the extract of orange fleshed sweet potato is able to ameliorate oxidative stress in a diseased model of insulin resistance. Assessment of the activity of antioxidant enzymes showed an increase in GPx activity but a decrease in CAT activity in palmitate treated groups. Treatment with the sweet potato extracts was able to reduce GPx activity and increase CAT activity. Furthermore, the extracts of Bophelo were also able to modulate the genes of these antioxidant enzymes. The expression level of both GPx and CAT decreased in the palmitate treated group and significantly improve upon treatments with extract. There was a significant reduction in the FRAP and TEAC status of palmitate treated groups and an improvement upon treatment with the extracts. This finding indicates that the extracts of sweet potato do not only have antioxidant ability but was also able to exhibit this activity in a diseased model of oxidative stress.

Glucose and fatty acid metabolism are important metabolic processes involved in the pathogenesis of type 2 diabetes (Randle, 1998; Boden, 2003). Therefore, the modulation of important genes and transcription factors involved in these two processes is another important strategy in the management of insulin resistance and type 2 diabetes. The extract of the orange fleshed sweet potato was able to influence the expression of GLUT 4, NRF1, MEF2A, ACC2 and CPT1 genes. There was a significant decrease in expression of GLUT4, NRF1 and MEF2A in the palmitate treated groups and a significant improvement upon treatment with the extracts. The expression level of these genes has been found to be directly correlated with glucose transport and glucose homeostasis in skeletal muscle. Disturbances in lipid metabolism and accumulated levels of intracellular fatty acids also contribute to the development of insulin resistance (Martins *et al.*, 2012). ACC2 and CPT1 are important genes involved in the regulation of fatty acid synthesis and oxidation (Wakil and Abu-Elheiga, 2009). There was a significant increase and decrease in the expression of ACC2 and CPT1 respectively in the palmitate treated group which was reversed upon treatments with the extracts. The modulation of these genes could possibly be due to the rich antioxidant profile and strong antioxidant activity of the orange fleshed sweet potato. Bophelo contained a relatively high amount of the flavonoids; hyperoside, quercetin and rutin. These compounds are antioxidant agents and their antidiabetic activities have been reported by different studies (Kamalakkannan and Prince, 2006; Kim *et al.*, 2011; Alam *et al.*, 2014; Ola *et al.*, 2015).

Generally, the leaves extract showed better improvement in all the parameters of oxidative stress measured. Similarly, the leaves' extract also modulate the expression level of all the genes determined more than the tubers. This correlates with the antioxidant profile and activity of the extracts, as the leaves of the orange fleshed sweet potato had higher amount of phenolic

compounds and also showed better antioxidant activity than the tubers. C2C12 cells treated with only extracts also improved across all the parameters measured. This suggests that the extracts are also able to offer protective effects in healthy cells by regulating intracellular antioxidant status and improving the expression level of the diabetes associated genes.

Another important strategy in the management of type 2 diabetes is the control of postprandial hyperglycemia through the inhibition of carbohydrate metabolizing enzymes. In this study, the leaves extract of orange fleshed sweet potato showed a better inhibition of alpha amylase, alpha glucosidase, sucrase and maltase than the tubers although not as much as acarbose. The study of Mai *et al.* (2007) demonstrated a positive correlation among alpha-glucosidase inhibitory activities, polyphenol contents and antioxidant activities in 24 edible plants. Therefore, the ability of the leaves' extracts to substantially inhibit the digestive enzymes in this study is possibly due to the higher phenolic contents.

In conclusion, this study provides information on the proximate composition, fatty acid composition and antioxidant profiles of seven South African grown cultivars of sweet potato. This information offers knowledge on the nutritional qualities of the selected sweet potato cultivars as well as possible areas for crop improvement. Nutrition therapy is an important aspect in the management of chronic diseases. Therefore, the consumption of nutritionally superior orange-fleshed cultivars should be encouraged for improved health and wellness. Furthermore, this study also demonstrated the antioxidant abilities and the antidiabetic potential of the extracts of the cultivars particularly the orange fleshed cultivar 'Bophelo'. The antioxidant activity and the anti-diabetic potentials of the extracts of the tubers and leaves of the orange fleshed cultivar can further be explored in the development of herbal drugs against type 2 diabetes.

7.2 RECOMMENDATION

This study has investigated the potential of sweet potato plant as a resource in the development of phytotherapy/herbal remedy against oxidative stress mediated insulin resistance. The effects of the extracts of the orange fleshed cultivar in an *in vivo* model of diabetes should be carried out to further corroborate the findings of this study. In addition, due to the fact that sweet potato is a food plant which is mostly consumed in the cooked form, *in vivo* studies on the effect of long term consumption in a condition of insulin resistance needs to be conducted. The effects of cooking processes on the presence and level of nutrients and beneficial phytochemicals are also necessary information that will be useful in exploring sweet potato as a functional food.

REFERENCES

Abdul-Ghani, M. A., & DeFronzo, R. A. (2010). Pathogenesis of insulin resistance in skeletal muscle. *BioMed Research International*, vol. 2010, Article ID 476279, 19 pages, doi:10.1155/2010/476279.

Adefegha, S. A., & Oboh, G. (2012). In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe^{2+}-induced lipid peroxidation in rat pancreas. *Asian Pacific Journal of Tropical Biomedicine*, 2(10), 774-781.

Adegbe, A. A., Larayetan R. A., & Omojuwa T. J. (2016). Proximate analysis, physicochemical properties and chemical constituents characterization of *Moringa Oleifera* (Moringaceae) seed oil using GC-MS analysis. *American Journal of Chemistry*, 6(2), 23-28.

Adisakwattana, S., & Chanathong, B. (2011). Alpha-glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract. *European Review for Medical and Pharmacological Sciences*, 15(7), 803-808.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry*, 51(26), 7825-7834.

Adsul, V. B., Khatiwora, E., Torane, R., & Deshpande, N. R. (2012). Antimicrobial activities of *Ipomoea carnea* leaves. *Journal of Natural Product and Plant Resources,* 2012; 2 (5), 597-600.

Ag, H. (1994). Pharmacology of α-glucosidase inhibition. *European Journal of Clinical Investigation*, 24(S3), 3-10.

Agbafor, K. N, Engwa, A. G., & Obiudu, I. K. (2015). Analysis of chemical composition of leaves and roots of *Ageratum conyzoides*. *International Journal of Current Research and Academic Review*, 3(11), 60-65.

Ahmed, F. N., Naqvi, F. N., & Shafiq, F. (2006). Lipid peroxidation and serum antioxidant enzymes in patients with type 2 diabetes mellitus. *Annals of the New York Academy of Sciences*, 1084(1), 481-489.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *invivo* and *invitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152.

Alam, M. M., Meerza, D., & Naseem, I. (2014). Protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. *Life Sciences*, 109(1), 8-14.

Alam, M. K., Rana, Z. H., & Islam, S. N. (2016). Comparison of the proximate composition, total carotenoids and total polyphenol content of nine orange-fleshed sweet potato varieties grown in Bangladesh. *Foods*, 5(3), 64.

Alayande, L. B., Mustapha, K. B., Dabak, J. D., & Ubom, G. A. (2012). Comparison of nutritional values of brown and white beans in Jos North local government markets. *African Journal of Biotechnology*, 11(43), 10135-10140.

Al-Farga, A., Zhang, H., Siddeeg, A., Shamoon, M., Chamba, M. V., & Al-Hajj, N. (2016). Proximate composition, functional properties, amino acid, mineral and vitamin contents of a novel food: Alhydwan (*Boerhavia elegana* Choisy) seed flour. *Food Chemistry*, 211, 268-273.

Amensour, M., Sendra, E., Abrini, J., Perez-Alvarez, J. A., & Fernandez-Lopez, J. (2010). Antioxidant activity and total phenolic compounds of myrtle extracts. *Journal of Food*, 8(2), 95-101.

Amoateng, P., Koffuor, G. A., Sarpong, K., & Agyapong, K. O. (2011). Free radical scavenging and anti-lipid peroxidative effects of a hydro-ethanolic extract of the whole plant of *Synedrella nodiflora* (L.) Gaertn (Asteraceae). *Free Radicals and Antioxidants*, 1(3), 70-78.

Anbuselvi, S, & Balamurugan, T. (2014). Phytochemical and antinutrient constituents of cassava and sweet potato. *World Journal of Pharmaceutical Science*, 2014; 3: 1440-1449.

Anderson, C. M., Hu, J., Barnes, R. M., Heidt, A. B., Cornelissen, I., & Black, B. L. (2015). Myocyte enhancer factor 2C function in skeletal muscle is required for normal growth and glucose metabolism in mice. *Skeletal Muscle*, 5(1), 7.

Angeloni, C., Pirola, L., Vauzour, D., & Maraldi, T. (2012). Dietary polyphenols and their effects on cell biochemistry and pathophysiology. *Oxidative Medicine and Cellular Longevity*, vol. 2012, Article ID 583901, 3 pages, doi:10.1155/2012/583901.

Anthoney Swamy, T., & Omwenga, J. (2014). Analysis of phytochemical composition of white and purple sweet potato (*Ipomoea batatas* [L] Lam) root. *Indian Journal of Advanced Plant Research* 2014; 1: 19-22.

Anthony, N. M., Sawi, M. K., Aiyelaagbe, O. O., Taiwo, A., Winnebah, T., & Fomba, S.N. (2014). Proximate characteristics and complementary assesment of five organic sweet potatoes cultivars and cowpea varieties. 3(9): 38-42.

Anuradha, C. V. (2013). Phytochemicals targeting genes relevant for type 2 diabetes. *Canadian Journal of physiology and pharmacology*, 91(6), 397-411.

Aouacheri, O., Saka, S., Krim, M., Messaadia, A., & Maidi, I. (2015). The investigation of the oxidative stress-related parameters in type 2 diabetes mellitus. *Canadian Journal of Diabetes*, 39(1), 44-49.

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectrum*, 17(3), 183-190.

Asha, B., Krishnamurthy, K., & Siddappa, D. (2011). Evaluation of anti-hyperglycaemic activity of *Zingiber officinale* (Ginger) in albino rats. *Journal of Chemical and Pharmaceutical Research*, 3, 452-456

Association of Official Analytical Chemists (AOAC 991.43) (2005a) 18th Edition, Chapter 32, pages 7-10.

Association of Official Analytical Chemists (AOAC 923.03) (2005b) 18th Edition, Chapter 32, page 2.

Association of Official Analytical Chemists (AOAC 996.06) (2005c) 18th edition, Chapter 41, pages 20-24.

Association of Official Analytical Chemists (AOACd) 17th edition, Chapter 32, pages 42 – 43.

Astawana, M., & Widowati, S. (2011). Evaluation of nutrition and glycemic index of sweet potatoes and its appropriate processing to hypoglycemic foods. *Indonesian Journal of Agricultural Science*, 12(1), 40-46.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, vol. 2014. Article ID 360438, 31 pages, doi:10.1155/2014/360438.

Ayeleso, T. B., Ramachela, K., & Mukwevho, E. (2016). A review of therapeutic potentials of sweet potato: Pharmacological activities and influence of the cultivar. *Tropical Journal of Pharmaceutical Research*, 15(12), 2751-2761.

Baar, K., Song, Z., Semenkovich, C. F., Jones, T. E., Han, D. H., Nolte, L. A., & Holloszy, J. O. (2003). Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *The FASEB Journal*, 17(12), 1666-1673.

Babbar, N., Oberoi, H. S., Sandhu, S. K., & Bhargav, V. K. (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. *Journal of Food Science and Technology*, 51(10), 2568-2575.

Babilas, P., Knie, U., & Abels, C. (2012). Cosmetic and dermatologic use of alpha hydroxy acids. *Journal of the German Society of Dermatology*, 10, 488–491.

Bajaj, S., & Khan, A. (2012). Antioxidants and diabetes. *Indian Journal of Endocrinology and Metabolism*, 16(Suppl 2), S267-S271.

Balbaa, M., Abdulmalek, S. A., & Khalil, S. (2017). Oxidative stress and expression of insulin signaling proteins in the brain of diabetic rats: Role of *Nigella sativa* oil and antidiabetic drugs. *PloS one*, 12(5), e0172429.

Barnard, R. J., Hong Gonzalez, J., Liva, M. E., & Ngo, T. H. (2006). Effects of a low-fat, high-fiber diet and exercise program on breast cancer risk factors in vivo and tumor cell growth and apoptosis *in vitro*. *Nutrition and Cancer*, 55(1), 28-34.

Barone, E., Calabrese, V., & Mancuso, C. (2009). Ferulic acid and its therapeutic potential as a hormetin for age-related diseases. *Biogerontology*, 10, 97-108.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70-76.

Bester, C., Van den Berg, A. A., & du Plooy, C. P. (1991). 'Blesbok' sweetpotato. Horticultural Science. 26, 1229-1230.

Bester C., & Louw J. H. (1992). Sweet potato breeding in South Africa. Vegetable and Ornamental Plant Institute. J. South African Society of Horticultural Science, 2, 22-68.

Boden, G., & Shulman, G. I. (2002). Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction. *European Journal of Clinical Investigation*, 32(S3), 14-23.

Boulé, N. G., Haddad, E., Kenny, G. P., Wells, G. A., & Sigal, R. J. (2001). Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *Jama*, 286(10), 1218-1227.

Bouwkamp, J. C., (1985). Sweet potato products: a natural resource for the tropics. CRC Press, Inc., Boca Raton, FL.

Brittain, H. G. (2001). Analytical profiles of drug substances and excipients. 28, 153-195.

Bruce, C. R., Hoy, A. J., Turner, N., Watt, M. J., Allen, T. L., Carpenter, K., & Kraegen, E. W. (2009). Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet–induced insulin resistance. *Diabetes*, 58(3), 550-558.

Bryan, N. S., Bian, K., & Murad, F. (2009). Discovery of the nitric oxide signaling pathway and targets for drug development. *Front Bioscience*, 14(1), 1-18.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, 52, 302-310.

Bugianesi, E., McCullough, A. J., & Marchesini, G. (2005). Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology*, 42(5), 987-1000.

Burattini, S., Ferri, P., Battistelli, M., Curci, R., Luchetti, F., & Falcieri, E. (2004). C2C12 murine myoblasts as a model of skeletal muscle development: morpho-functional characterization. *European Journal of Histochemistry*, 48(3), 223-233.

Burén, J., Lindmark, S., Renström, F., & Eriksson, J. W. (2003). *In vitro* reversal of hyperglycemia normalizes insulin action in fat cells from type 2 diabetes patients: is cellular insulin resistance caused by glucotoxicity in vivo? *Metabolism*, 52(2), 239-245.

Burn, P., & Kishore, G. M. (2000). Food as a source of health enhancing compounds. *AgBioForum* 3(1), 3-9,

Buse, M. G. (2006). Hexosamines, insulin resistance, and the complications of diabetes: current status. *American Journal of Physiology-Endocrinology and Metabolism*, 290(1), E1-E8.

Cahova, M., Vavrinkova, H., & Kazdova, L. (2007). Glucose-fatty acid interaction in skeletal muscle and adipose tissue in insulin resistance. *Physiological Research*, 56(1), 1-15.

Calabrese, V., Cornelius, C., Leso, V., Trovato-Salinaro, A., Ventimiglia, B., Cavallaro, M., & Castellino, P. (2012). Oxidative stress, glutathione status, sirtuin and cellular stress response in type 2 diabetes. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1822(5), 729-736.

Carpena, A.L. (2009). Important cultivars, varieties, and hybrids. In the sweet potato. Springer Netherlands; pages 27-40.

Cai, X., Han, X., Luo, Y., & Ji, L. (2013). Comparisons of the efficacy of alpha glucosidase inhibitors on type 2 diabetes patients between Asian and Caucasian. *PloS one*, 8(11), e79421.

Chakrabarti, R., & Rajagopalan, R. (2002). Diabetes and insulin resistance associated disorders: disease and the therapy. *Current Science*, 1533-1538.

Chandrasekara, A., Kumar, T.J. (2016). Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. *International Journal of Food Science,* Vol. 2016, Article ID 3631647, 15 pages, http://dx.doi.org/10.1155/2016/3631647.

Chang WH, Chen CM, Hu SP, Kan NW, Chiu CC, Liu JF. (2007). Effect of purple sweet potato leaves consumption on the modulation of the immune response in basketball players during the training period. *Asia Pacific Journal of Clinical Nutrition*, 16: 609-615.

Chang, C. I., Hsu, C. M., Li, T. S., Huang, S. D., Lin, C. C., Yen, C. H., & Cheng, H. L. (2014). Constituents of the stem of *Cucurbita moschata* exhibit antidiabetic activities through multiple mechanisms. *Journal of Functional Foods*, 10, 260-273.

Che, M., Wang, R., Li, X., Wang, H. Y., & Zheng, X. S. (2016). Expanding roles of superoxide dismutases in cell regulation and cancer. *Drug Discovery Today*, 21(1), 143-149.

Chen, C. M., Li, S. C., Liao, Y. Y., Liu, J. F., Shyur, L. F., Chen, P. R. & Chen, C. Y. (2013). *Invitro* immunomodulatory effect of *Ipomoea batatas* L. tuber bioactives. *The FASEB J,* 27(1 Supplement): 862-869.

Chen, Y. C., Chang, H. S., Lai, H. M., & Jeng, S. T. (2005). Characterization of the wound inducible protein IPOMOELIN from sweet potato. *Plant Cell Environment*, 28, 251-259.

Chivenge, P., Mabhaudhi, T., Modi, A. T. & Mafongoya, P. (2015). The potential role of neglected and underutilised crop species as future crops under water scarce conditions in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health*, 12(6), 5685-5711.

Choi, C. S., Savage, D. B., Abu-Elheiga, L., Liu, Z. X., Kim, S., Kulkarni, A., & Zhang, D. (2007). Continuous fat oxidation in acetyl–CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. *Proceedings of the National Academy of Sciences*, 104(42), 16480-16485.

CIP (1995). Sweet potato, treasure for the poor. La Molina, Peru: International Potato centre. Available from http://www.cipotato.org/sweetpotato/ on December 4, 2007.

DeFronzo, R. A., & Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*, 32(suppl 2), S157-S163.

Deguchi, Y., Osada, K., Uchida, K., Kimura, H., Yoshikawa, M., Kudo, T., & Watanuki, M. (1998). Effects of extract of guava leaves on the development of diabetes in the db/db mouse and on the postprandial blood glucose of human subjects. *Journal of the Agricultural Chemical Society of Japan*, 72(8), 923-931.

Department of Agriculture, Forestry and Fisheries, Republic of South Africa (2011) Sweet potato production retrieved from http://www.nda.agric.za/docs/Brochures/PG_SweetPotato.pdf.

Deponte, M. (2013). Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830(5), 3217-3266.

Devasagayam, T.P.A., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., & Lele, R.D. (2004). Free radicals and antioxidants in human health: current status and future prospects. The *Journal of the Association of Physicians in India,* 52, 794-804.

Dewi, R. T., & Maryani, F. (2015). Antioxidant and α-Glucosidase Inhibitory Compounds of Centella Asiatica. *Procedia Chemistry*, 17, 147-152.

Dhakar R, Maurya S, Pooniya B, Bairwa N, & Gupta M. (2011). Moringa, The herbal gold to combat malnutrition. *Chronicles of Young Scientists*, 2(3), 119-125.

Dhanya, R., Arun, K. B., Syama, H. P., Nisha, P., Sundaresan, A., Kumar, T. S., & Jayamurthy, P. (2014). Rutin and quercetin enhance glucose uptake in L6 myotubes under oxidative stress induced by tertiary butyl hydrogen peroxide. *Food chemistry*, 158, 546-554.

Doehner, W., Gathercole, D., Cicoira, M., Krack, A., Coats, A. J., Camici, P. G., & Anker, S. D. (2010). Reduced glucose transporter GLUT4 in skeletal muscle predicts insulin resistance in non-diabetic chronic heart failure patients independently of body composition. *International Journal of Cardiology*, 138(1), 19-24.

Dsouza, D. O. R. I. N., & Lakshmidevi, N. (2015). Models to study *in vitro* antidiabetic activity of plants: A review. *International Journal of Pharma and Bio Sciences,* 732-741.

Dunlop, M. (2000). Aldose reductase and the role of the polyol pathway in diabetic nephropathy. *Kidney International*, 58, S3-S12.

Dutta, S. (2015). Sweet potatoes for diabetes mellitus: A systematic review. *Pharmacophore*, 6, 60-72.

Dworatzek, P. D., Arcudi, K., Gougeon, R., Husein, N., Sievenpiper, J. L., & Williams, S. L. (2013). Nutrition therapy. *Canadian Journal of Diabetes*, 37, S45-S55.

Eastwood, M. A. (1999). Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? *Monthly Journal of the Association of Physicians*, 92(9), 527-530.

Efird, J. T., Choi, Y. M., Davies, S. W., Mehra, S., Anderson, E. J., & Katunga, L. A. (2014). Potential for improved glycemic control with dietary *Momordica charantia* in patients with insulin resistance and pre-diabetes. *International Journal of Environmental Research and Public Health*, 11(2), 2328-2345.

El-Aal, H. A. H. M. A. (2012). Lipid peroxidation end-products as a key of oxidative stress: Effect of antioxidant on their production and transfer of free radicals. In lipid peroxidation. book edited by Angel Catala, ISBN 978-953-51-0716-3.

El-Bahr, S. M. (2013). Curcumin regulates gene expression of insulin like growth factor, B-cell CLL/lymphoma 2 and antioxidant enzymes in streptozotocin induced diabetic rats. *BMC Complementary and Alternative Medicine,* 13(1), 368, doi: 10.1186/1472-6882-13-368.

Elmezian H, Zawawy FM, EL-Helbawy S, Mostafa M, El Aily M and Ayat Mosleh (2016). Separation and identification of the components of *Acacia sieberiana* stem bark plant extract and study its safety as a treatment drug for diarrhea. *Analytical Chemistry: An Indian Journal.* 16 (13).

Eriksson, K. F., & Lindgärde, F. (1991). Prevention of Type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise .The 6-year Malmö feasibility study. *Diabetologia*, 34(12), 891-898.

Etxeberria, U., de la Garza, A. L., Campión, J., Martinez, J. A., & Milagro, F. I. (2012). Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert Opinion on Therapeutic Targets*, 16(3), 269-297.

Evans, J. L., Maddux, B. A., & Goldfine, I. D. (2005). The molecular basis for oxidative stress-induced insulin resistance. *Antioxidants & Redox Signaling*, 7(7-8), 1040-1052.

FAO (1990). Production Statistics for 1990. *FAO*, Rome, Italy.

Fawole, O. A., & Opara, U. L. (2016). Stability of total phenolic concentration and antioxidant capacity of extracts from pomegranate co-products subjected to *in vitro* digestion. *BMC Complementary and Alternative Medicine*, 16(1), 358, doi: 10.1186/s12906-016-1343-2.

Ferreira, Z., Ayeleso, A., & Mukwevho, E. (2014). Control of carbohydrate and lipid metabolism by NRF-1 and sirtuins: Implications on type 2 diabetes and obesity. *Chemical Biology Letters*, 1(2), 66-76.

Ferris, R. S. B., Wanda, K., Tuyisenge, J., Munkankubana, D., Kagiraneza, B., Ngaboyisonga, C., Gatarayiha, C., Uwantege, C., Kanyange, C., & Butare, L. (2002). Sweetpotato Sub-sector market survey Rwanda, ATDT-CIAT/ISAR/IITA-FOODNET and PEARL-Project, Rwanda,

Fidrianny, I., Windyaswari, A. S., & Wirasutisna, K. R. (2013). Antioxidant capacities of various leaves extract from five colors varieties of sweet potatoes tubers using ABTS, DPPH assays and correlation with total flavonoid, phenolic, carotenoid content. *Research Journal of Medicinal Plant*, 7(3), 130-140.

Florence TM (1995). The role of free radicals in disease. *Australian and New Zealand Journal of Opthamology*, 23(1), 3-7.

NWU
LIBRARY

Flores, G., Wu, S. B., Negrin, A., & Kennelly, E. J. (2015). Chemical composition and antioxidant activity of seven cultivars of guava (*Psidium guajava*) fruits. *Food Chemistry*, 170, 327-335.

Food and Agriculture Organization (FAO) of the United Nations (2015). Food and Agriculture Organization Statistical Databases (FAOSTAT).

Fu, Z. F., Tu, Z. C., Zhang, L., Wang, H., Wen, Q. H., & Huang, T. (2016). Antioxidant activities and polyphenols of sweet potato (*Ipomoea batatas* L.) leaves extracted with solvents of various polarities. *Food Bioscience*, 15, 11-18.

Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., & Ogihara, T. (2005). Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism*, 54(3), 387-390.

Gaikwad, S. B., Mohan, G. K., & Rani, M. S. (2014). Phytochemicals for diabetes management. *Pharmaceutical Crops*, 31(2), 261-266.

Garcia, Y. J., Rodríguez-Malaver, A. J., & Peñaloza, N. (2005). Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. *Journal of Neuroscience Methods*, 144(1), 127-135.

Gawlik, K., Naskalski, J. W., Fedak, D., Pawlica-Gosiewska, D., Grudzień, U., Dumnicka, P., & Solnica, B. (2015). Markers of antioxidant defense in patients with type 2 diabetes. *Oxidative medicine and cellular longevity*, vol. 2016, Article ID 2352361, 6 pages, 2016. doi:10.1155/2016/2352361.

Ghasemzadeh, A., & Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, 5(31), 6697-6703.

Ghasemzadeh, A., Omidvar, V., & Jaafar, H. Z. (2012). Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas*). *Journal of Medicinal Plants Research*, 6(15), 2971-2976.

Giugliano, D., Ceriello, A., & Esposito, K. (2008). Glucose metabolism and hyperglycemia. *The American Journal of Clinical Nutrition*, 87(1), 217S-222S.

Godswill, N.N., Martin, B.J., Kingsley, T.M., Albert, D.M.J., Thierry, K.S., Sastile, M.N., Hermine, N.B. & Emmanuel, Y. (2016). Effects of Dietary Fatty Acids on Human Health: Focus on Palm oil from Elaeis guineensis Jacq. and Useful Recommendations. *Food and Public Health*, *6*(3), 75-85.

Gogtay, N. J., & Thatte, U. M. (2017). Principles of Correlation analysis. *Journal of the Association of Physicians of India*, 65, 78.

Grace, M. H., Yousef, G. G., Gustafson, S. J., Truong, V. D., Yencho, G. C., & Lila, M. A. (2014). Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweetpotato storage and impacts on bioactive properties. *Food chemistry*, 145, 717-724.

Gripper J. (2013). "Sweet Potato." Vegetable Book. Forgotten Natural Remedies. S.l.: Lulu Com, pages 87-88.

Grosso, G., Bei, R., Mistretta, A., Marventano, S., Calabrese, G., Masuelli, L., & Gazzolo, D. (2013). [Frontiers in Bioscience 18, 1017-1029, June 1, 2013] Effects of Vitamin C on health: a review of evidence. *Frontiers in Bioscience*, 18, 1017-1029.

Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, 217(2), 213-220.

Gumede, D. B., & Ojuka, E. O. (2011). NRF-1 overexpression increases GLUT4 via MEF2A. The *FASEB Journal*, 25(1 Supplement), 1038.20.

Guo, X., Yoshitomi, H., Gao, M., Qin, L., Duan, Y., Sun, W., & Liu, T. (2013). Guava leaf extracts promote glucose metabolism in SHRSP. Z-Leprfa/Izm rats by improving insulin resistance in skeletal muscle. *BMC Complementary and Alternative Medicine*, 13(1), 52. doi: 10.1186/1472-6882-13-52.

Gurmu F, Hussein S, & Laing M. (2014). The potential of orange-fleshed sweet potato to prevent vitamin A deficiency in Africa. *International Journal for Vitamin and Nutrition Research*, 84, 65-78.

Hadwan, M. H., & Abed, H. N. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. *Data in Brief*, 6, 194-199.

Halawa, I., Zelano, J., & Kumlien, E. (2015). Hypoglycemia and risk of seizures: a retrospective cross-sectional study. *Seizure*, *25*, 147-149.

Hamza, A. M., Collins, A., Ado, S. G., Ikuenobe, C. E., Ataga, C. D., & Odewale, J. O. (2014). Proximate compositions evaluation and variability among cultivars of date palm (*Phoenix dactylifera* L.) in Nigeria. *International Journal of Plant & Soil Science*, 3(3), 248-259.

Hanieh, H., Gerile, C., Narabara, K., Gu, Z., Abe, A., Kondo, Y. (2010). *In vivo* immunomodulatory effects of dietary purple sweet potato after immunization in chicken. *Journal of Animal Science*, 81, 116-121.

Hara, Y., & Honda, M. (1990). The inhibition of α-amylase by tea polyphenols. *Agricultural and Biological Chemistry*, 54(8), 1939-1945.

Harriet Wallberg-Henriksson, J. R. Z. (2001). GLUT4: a key player regulating glucose homeostasis? Insights from transgenic and knockout mice. *Molecular Membrane Biology*, 18(3), 205-211.

Harrison, H. F., Peterson, J. K., Snook, M. E., Bohac, J. R., & Jackson, D. M. (2003). Quantity and potential biological activity of caffeic acid in sweet potato [*Ipomoea batatas* (L.) Lam.] storage root periderm. *Journal of Agricultural and Food Chemistry*, 51(10), 2943-2948.

Hassan, B. A. R. (2013). Overview on diabetes mellitus (type 2). *Journal of Chromatography and Separation Techniques*, 4(2), http://dx.doi.org/10.4172/2157-7064.1000e114.

Henriksen, E. J., Diamond-Stanic, M. K. & Marchionne, E. M. (2011). Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radical Biology and Medicine*, 51, 993-999.

Hermes, D., Dudek, D. N., Maria, M. D., Horta, L. P., Lima, E. N., de Fátima, Â., Sanches, A. C., Modolo, L. V. (2013). *In vivo* wound healing and antiulcer properties of white sweet potato (*Ipomoea batatas*). *Journal of Advanced Research*, 4, 411-415.

Hijmans, R. J., Huaccho, L., & Zhang, D.P. (2000). Distribution of sweet potato. CIP Program Report 1999, 323-329.

Hirabara, S. M., Curi, R., & Maechler, P. (2010). Saturated fatty acid-induced insulin resistance is associated with mitochondrial dysfunction in skeletal muscle cells. *Journal of Cellular Physiology*, 222(1), 187-194.

Houstis, N., Rosen, E. D., & Lander, E. S. (2006). Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*, 440(7086), 944-948.

Huang, S., & Czech, M. P. (2007). The GLUT4 glucose transporter. *Cell Metabolism*, 5(4), 237-252.

Huang, W. Y., Cai, Y. Z., & Zhang, Y. (2009). Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and Cancer*, 62(1), 1-20.

Hue, S. M., Chandran, S., & Boyce, A. N. (2010). Variation of leaf and storage roots morphology on *Ipomoea batatas* L. (Sweet potato) cultivars. *Asia Pacific Symposium on Postharvest Research, Education and Extension*, 943: 73-79.

Hue S-W, Low MY (2015). An insight into sweet potato weevils management: a review," Psyche, vol. 2015, Article ID 849560, 11 pages, 2015. doi:10.1155/2015/849560.

Hue, S. M., Boyce, A. N. & Chandran, S. (2012). Antioxidant activity, phenolic and flavonoid contents in different varieties of sweet potato (*Ipomea batatas*). *Australian Journal of Crop Science*, 6, 375-380

Ijuin, T., & Takenawa, T. (2012). Regulation of insulin signaling and glucose transporter 4 (GLUT4) exocytosis by phosphatidylinositol 3, 4, 5-trisphosphate (PIP3) phosphatase, skeletal muscle, and kidney enriched inositol polyphosphate phosphatase (SKIP). *Journal of Biological Chemistry*, 287(10), 6991-6999.

Ingabire, M. R. & Hilda, V. (2011). Comparison of the nutrient composition of four sweet potato varieties cultivated in Rwanda. *American Journal of Food and Nutrition*, 1(1), 34-38.

Islam, M. J., Haque, M. Z., Majunder, K., Haque, M., & Hossain, F. (2002). Growth and yield potential of nine selected genotypes of sweet potato. *Pakistan Journal of Biological Sciences*, 5(5), 537-538.

Islam, M. S., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., & Yamakawa, O. (2002). Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas L.*) genotypes. *Journal of Agricultural and Food Chemistry*, 50(13), 3718-3722.

Islam, S. (2014). Nutritional and medicinal qualities of sweet potato tops and leaves. Cooperative Extension Service, University of Arkansas, https://www.uaex.edu/publications/PDF/FSA-6135.pdf.

Islam, S. N., Nusrat, T., Begum, P., & Ahsan, M. (2016). Carotenoids and β-carotene in orange fleshed sweet potato: A possible solution to vitamin A deficiency. *Food chemistry*, 199, 628-631.

Jeeva, J. S., Sunitha, J., Ananthalakshmi, R., Rajkumari, S., Ramesh, M., & Krishnan, R. (2015). Enzymatic antioxidants and its role in oral diseases. *Journal of Pharmacy & Bioallied Sciences*, 7(Suppl 2), S331-S333.

Jeong, B. G., Chun, J., Jung, J., Islam, M. A., Sharmin, F., Quines, V., & Choi, S. G. (2017). Variations in vitamin E, phenolic content, and antioxidant properties of different wheat cultivars of South Korea. CyTA, *Journal of Food*, 1-5.

Ji, H., Zhang, H., Li, H., & Li, Y. (2015). Analysis on the nutrition composition and antioxidant activity of different types of sweet potato cultivars. *Food Nutrition and Science*, 6, 161.

Joshi, D., Patel, H., Baker, D. M., Shiwen, X., Abraham, D. J., & Tsui, J. C. (2011). Development of an *in vitro* model of myotube ischemia. *Laboratory Investigation*, 91(8), 1241-1252.

Kabel, A. M. (2014). Free radicals and antioxidants: role of enzymes and nutrition. *World Journal of Nutrition and Health*, 2(3), 35-38.

Kalkan, I. H., & Suher, M. (2013). The relationship between the level of glutathione, impairment of glucose metabolism and complications of diabetes mellitus. *Pakistan Journal of Medical Sciences*, 29(4), 938-942.

Kamakura, R., Son, M. J., de Beer, D., Joubert, E., Miura, Y., & Yagasaki, K. (2015). Antidiabetic effect of green rooibos (*Aspalathus linearis*). *Cytotechnology*, 67(4), 699-710.

Kamalakkannan, N., & Prince, P. S. M. (2006). Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. *Basic & Clinical Pharmacology & Toxicology*, 98(1), 97-103.

Kampmann, U., Christensen, B., Nielsen, T. S., Pedersen, S. B., Orskov, L., Lund, S., & Jessen, N. (2011). GLUT4 and UBC9 protein expression is reduced in muscle from type 2 diabetic patients with severe insulin resistance. *PloS one*, 6(11), e27854.

Kang, H., Kwak, Y., & Koppula, S. (2014). Protective effect of purple sweet potato (*Ipomoea batatas* Linn, Convolvulaceae) on neuroinflammatory responses in lipopolysaccharide-stimulated microglial cells. *Tropical Journal of Pharmaceutical Research*, 13(8), 1257-1263.

Kapinga, R., Ortiz, O., Ndunguru, J., Omiat, E., Tumwegamire, S. (2007). Handbook of Sweet potato integrated crop management. Research outputs and programs for East Africa (1995-2006).

Karna, P., Gundala, S. R., Gupta, M. V., Shamsi, S. A., Pace, R. D., Yates, C., & Aneja, R. (2011). Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells *in vitro* and *in vivo*. *Carcinogenesis*, 32(12), 1872-1880.

Kaskoos, R. A. (2013). *In vitro* α-glucosidase inhibition and antioxidant activity of methanolic extract of *Centaurea calcitrapa* from Iraq. *American Journal of essential Oils and Natural Products*, 1, 122-125.

Katalinić, V., Možina, S. S., Skroza, D., Generalić, I., Abramovič, H., Miloš, M., & Boban, M. (2010). Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chemistry*, 119(2), 715-723.

Kazeem, M. I., & Ashafa, A. O. T. (2015). *In vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. *Journal of Herbal Medicine*, 5(3), 158-164.

Kazeem, M. I., & Davies, T. C. (2016). Anti-diabetic functional foods as sources of insulin secreting, insulin sensitizing and insulin mimetic agents. *Journal of Functional Foods*, 20, 122-138.

Kazeem, M. I., Abimbola, S. G., & Ashafa, A. O. T. (2013). Inhibitory potential of *Gossypium arboreum* leaf extracts on diabetes key enzymes, α-amylase and α-glucosidase. *Bangladesh Journal of Pharmacology*, 8(2), 149-155.

Keane, K. N., Cruzat, V. F., Carlessi, R., de Bittencourt, P. I. H., & Newsholme, P. (2015). Molecular events linking oxidative stress and inflammation to insulin resistance and β-cell dysfunction. *Oxidative medicine and cellular longevity*, 2015:181643. doi: 10.1155/2015/181643.

Kennedy, G., & Burlingame, B. (2003). Analysis of food composition data on rice from a plant genetic resources perspective. *Food Chemistry*, *80*(4), 589-596.

Kerio, L. C., Wachira, F. N., Wanyoko, J. K., & Rotich, M. K. (2013). Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chemistry*, 136(3), 1405-1413.

Kerksick, C., & Willoughby, D. (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*, 2(2), 38.

Kifayatullah, M., & Sengupta, P. (2016). Effect of *Pericampylus glaucus* on plasma glucose concentration and lipid profile in streptozotocin-induced diabetic rats. *Bangladesh Journal of Pharmacology*, 11(1), 200-205.

Kim, J. M., Park, S. J., Lee, C. S., Ren, C., Kim, S. S., & Shin, M. (2011). Functional properties of different Korean sweet potato varieties. *Food Science and Biotechnology*, *20*(6), 1501-1507.

Kim, O. K., Nam, D. E., Yoon, H. G., Baek, S. J., Jun, W., & Lee, J. (2015). Immunomodulatory and antioxidant effects of purple sweet potato extract in LP-BM5 murine leukemia virus-induced murine acquired immune deficiency syndrome. *Journal of Medicinal food*, 18(8), 882-889.

Kim, H. P., Son, K. H., Chang, H. W., & Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences*, 96(3), 229-245.

Kim, J. H., Kang, M. J., Choi, H. N., Jeong, S. M., Lee, Y. M., & Kim, J. I. (2011). Quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. *Nutrition Research and Practice*, 5(2), 107-111.

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y., & Rhee, H. I. (2005). Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. *Nutrition*, 21(6), 756-761.

Konczak-Islam, I., Yoshimoto, M., Hou, D. X., Terahara, N., & Yamakawa, O. (2003). Potential chemopreventive properties of anthocyanin-rich aqueous extracts from *in vitro* produced tissue

of sweet potato (*Ipomoea batatas* L.). *Journal of Agricultural and Food Chemistry*, 51(20), 5916-5922.

Ku, K. M., Kim, H. S., Kim, S. K., & Kang, Y. H. (2014). Correlation analysis between antioxidant activity and phytochemicals in Korean colored corns using principal component analysis. *Journal of Agricultural Science*, 6(4), doi: https://doi.org/10.5539/jas.v6n4p1.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, vol. 2013, Article ID 162750, 16 pages, 2013. doi:10.1155/2013/162750.

Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2011). α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacognosy Reviews*, 5(9), 19.

Kusano, S., & Abe, H. (2000). Antidiabetic activity of white skinned sweet potato (*Ipomoea batatas* L.) in obese Zucker fatty rats. *Biological and Pharmaceutical Bulletin*, 23(1), 23-26.

Kwon, Y. I., Apostolidis, E., & Shetty, K. (2008). Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry*, 32(1), 15-31.

Laurie, S.M., & Niederwieser, J.G. (2004). The Sweet potato plant. In: Niederwieser JG editor. Guide to sweet potato production in South Africa. ARC-Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, South Africa, 7-14.

Laurie, S., Calitz, F., Mtileni, M., Mphela, W., & Tjale, S. (2017). Performance of informal market sweet potato cultivars in on-farm trials in South Africa. *Open Agriculture*, 2(1), 431-441.

Le Lay, S., Simard, G., Martinez, M. C., & Andriantsitohaina, R. (2014). Oxidative stress and metabolic pathologies: from an adipocentric point of view. *Oxidative Medicine and Cellular Longevity*, vol. 2014, Article ID 908539, 18 pages, 2014. doi:10.1155/2014/908539.

Lebovitz, H. E. (1999). Type 2 diabetes: an overview. *Clinical Chemistry*, 45(8), 1339-1345.

Lee, S. L., Chin, T. Y., Tu, S. C., Wang, Y. J., Hsu, Y. T., Kao, M. C., & Wu, Y. C. (2015). Purple sweet potato leaf extract induces apoptosis and reduces inflammatory adipokine expression in 3T3-L1 differentiated adipocytes. *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 126302, 9 pages, 2015. doi:10.1155/2015/126302.

Lee, H. H., Kang, S. G. & Rhim, J. W. (1999). Characteristics of antioxidative and antimicrobial activities of various cultivars of sweet potato. *Korean Journal of Food Science and Technology*, 31, 1090-1095.

Legua, P., Melgarejo, P., Abdelmajid, H., Martínez, J. J., Martínez, R., Ilham, H., & Hernández, F. (2012). Total phenols and antioxidant capacity in 10 Moroccan pomegranate varieties. *Journal of Food Science*, 77(1), C115-C120.

Li, P. G., Mu, T. H., & Deng, L. (2013). Anticancer effects of sweet potato protein on human colorectal cancer cells. *World Journal of Gastroenterology*, 19(21), 3300-8. doi: 10.3748/wjg.v19.i21.3300.

Lila, M. A. (2004). Anthocyanins and human health: an *in vitro* investigative approach. *BioMed Research International*, 2004(5), 306-313.

Lindsay, R. S., Funahashi, T., Hanson, R. L., Matsuzawa, Y., Tanaka, S., Tataranni, P. A., & Krakoff, J. (2002). Adiponectin and development of type 2 diabetes in the Pima Indian population. *The Lancet*, 360(9326), 57-58.

Liu, C. W., Wang, Y. C., Hsieh, C. C., Lu, H. C., & Chiang, W. D. (2015). Guava (*Psidium guajava* Linn.) leaf extract promotes glucose uptake and glycogen accumulation by modulating the insulin signaling pathway in high-glucose-induced insulin-resistant mouse FL83B cells. *Process Biochemistry*, 50(7), 1128-1135.

Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M., & Chou, C. J. (2008). Flavonoids for controlling starch digestion: structural requirements for inhibiting human α-amylase. *Journal of Medicinal Chemistry*, 51(12), 3555-3561.

Lopaschuk, G. D. (2016). Fatty acid oxidation and its relation with insulin resistance and associated disorders. *Annals of Nutrition and Metabolism*, 68(Suppl. 3), 15-20.

Lorenzi, M. (2007). The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Journal of Diabetes Research*, 2007:61038. doi: 10.1155/2007/61038.

Lu, S. C. (2009). Regulation of glutathione synthesis. *Molecular Aspects of Medicine*, 30(1), 42-59.

Ludvik, B., Hanefeld, M., & Pacini, G. (2008). Improved metabolic control by *Ipomoea batatas* (Caiapo) is associated with increased adiponectin and decreased fibrinogen levels in type 2 diabetic subjects. *Diabetes, Obesity and Metabolism*, 10(7), 586-592.

Ludvik, B., Neuffer, B., & Pacini, G. (2004). Efficacy of *Ipomoea batatas* (Caiapo) on diabetes control in type 2 diabetic subjects treated with diet. *Diabetes Care*, *27*(2), 436-440.

Ludvik, B. H., Mahdjoobian, K., Waldhaeusl, W., Hofer, A., Prager, R., Kautzky-Willer, A. & Pacini, G. (2002). The Effect of *Ipomoea batatas* (Caiapo) on glucose metabolism and serum cholesterol in patients with type 2 diabetes a randomized study. *Diabetes Care*, 25, 239-240

Luka, C. D., Olatunde, A., Tijjani, H. & Olisa-Enewe, I. A. (2013). Effect of aqueous extract of *Phaseolus vulgaris* L. (Red Kidney Beans) on alloxan-induced diabetic wistar rats. *International Journal of Science Inventions Today*, 2, 292-301.

Lushchak, V. I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *Journal of Amino Acids*, vol. 2012. Article ID 736837, 26 pages, doi:10.1155/2012/736837.

Magiera, S., & Zaręba, M. (2015). Chromatographic determination of phenolic acids and flavonoids in *Lycium barbarum* L. and evaluation of antioxidant activity. *Food Analytical Methods*, 8(10), 2665-2674.

Mai, T. T., Thu, N. N., Tien, P. G., & Van Chuyen, N. (2007). Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of Nutritional Science and vitaminology*, 53(3), 267-276.

Makhafola, T. J., Elgorashi, E. E., McGaw, L. J., Verschaeve, L., & Eloff, J. N. (2016). The correlation between antimutagenic activity and total phenolic content of extracts of 31 plant species with high antioxidant activity. *BMC Complementary and Alternative Medicine*, 16(1), 490, doi: 10.1186/s12906-016-1437-x.

Maksimovic, Z., Malencic, D., & Kovacevic, N., (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. *Bioresource Technology*, 8, 873-877.

Maritim, A. C., Sanders, A., & Watkins, J. 3. (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology*, 17(1), 24-38.

Martins, A. R., Nachbar, R. T., Gorjao, R., Vinolo, M. A., Festuccia, W. T., Lambertucci, R. H., & Hirabara, S. M. (2012). Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. *Lipids in Health and Disease*, 11:30. doi: 10.1186/1476-511X-11-30.

Masek, A., Chrzescijanska, E., & Latos, M. (2016). Determination of antioxidant activity of caffeic acid and p-coumaric acid by using electrochemical and spectrophotometric assays. *International Journal of Electrochemical Science*, 11(12), 10644-10658.

Mathebula, S. D. (2015). Polyol pathway: A possible mechanism of diabetes complications in the eye. *African Vision and Eye Health*, 74(1), 5 pages. http://dx.doi.org/10.4102/ aveh.v74i1.13.

Mayes, S., Massawe, F.J., Alderson, P.G., Roberts, J.A., Azam-Ali, S.N. & Hermann, M., 2011. The potential for underutilized crops to improve security of food production. *Journal of Experimental Botany*, 63(3), 1075-1079.

Mazibuko, S. E., Muller, C. J. F., Joubert, E., De Beer, D., Johnson, R., Opoku, A. R., & Louw, J. (2013). Amelioration of palmitate-induced insulin resistance in C2C12 muscle cells by rooibos (*Aspalathus linearis*). *Phytomedicine*, 20(10), 813-819.

Mazid, M., Khan, T. A., Khan, Z. H., Quddusi, S., & Mohammad, F. (2011). Occurrence, biosynthesis and potentialities of ascorbic acid in plants. *International Journal of Plant, Animal and Environmental Sciences*, 1(2), 167-184.

Mazza G. (2007). Anthocyanins and heart health. *Ann 1st Super Sanita*, 43(4), 369-374.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., & Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *Journal of Agricultural and Food Chemistry*, 47(10), 4009-4017.

Mbaeyi-Nwaoha, I. E & Emejulu, V. N. (2013). Evaluation of phytochemical composition and antimicrobial activity of sweet potato (*Ipomoea batatas*) leaf. *Pakistan Journal of Nutrition, 12, 575-586.*

McCue, P. P., & Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pacific Journal of Clinical Nutrition*, 13(1), 101-106.

McGee, S. L., & Hargreaves, M. (2006). Exercise and skeletal muscle glucose transporter 4 expression: Molecular mechanisms. *Clinical and Experimental Pharmacology and Physiology*, 33(4), 395-399.

McGee, S. L., Sparling, D., Olson, A. L., & Hargreaves, M. (2006). Exercise increases MEF2-and GEF DNA-binding activity in human skeletal muscle. *The FASEB journal*, 20(2), 348-349.

Mendonça-Filho R. R. (2006). Bioactive phytocompounds: New approaches in the phytosciences. In: I. Ahmad, F. Aqil, M. Owais, editors. Modern phytomedicine: Turning medicinal plants into drugs, 1-25.

Miao, M., Jiang, H., Jiang, B., Zhang, T., Cui, S. W., & Jin, Z. (2014). Phytonutrients for controlling starch digestion: Evaluation of grape skin extract. *Food Chemistry*, 145, 205-211.

Miled, H. B., Saada, M., Jallali, I., Barka, Z. B., Tlili, M., Alimi, H., & Tebourbi, O. (2017). Variability of antioxidant and biological activities of *Rhus tripartitum* related to phenolic compounds. *EXCLI journal*, 16, 439–447.

Mogale, M. A., Lebelo, S. L., Thovhogi, N., De Freitas, A. N., & Shai, L. J. (2011). α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [(A. Rich.) Hochst.] subspecies caffra (Sond) Kokwaro (Anacardiaceae) stem-bark extracts. *African Journal of Biotechnology*, 10(66), 15033-15039.

Montejo, J. F., Mondonedo, J. A. B., Lee, M. G. A., Ples, M. B., & Vitor, R. J. S. (2015). Hematological effects of *Ipomoea batatas* (camote) and *Phyllanthus niruri* (sampa-sampalukan) from Philippines in the ICR mice (Mus musculus). *Asian Pacific Journal of Tropical Biomedicine*, *5*(1), 29-33.

Montilla, E. C., Hillebrand, S., & Winterhalter, P. (2011). Anthocyanins in purple sweet potato (*Ipomoea batatas* L.) varieties. *Fruit, Vegetable and Cereal Science and Biotechnology*, 5(2), 19-23.

Morales-González, J. A. (2013). Oxidative stress and chronic degenerative diseases-a role for antioxidants. Rijeka: Croatia InTech, Edited by José A. Morales-González, ISBN 978-953-51-1123-8, 512 pages, Publisher: InTech, Chapters published May 22, 2013 DOI: 10.5772/45722.

Mytilineou, C., Kramer, B. C., & Yabut, J. A. (2002). Glutathione depletion and oxidative stress. *Parkinsonism & Related Disorders*, 8(6), 385-387.

Nagai, M., Tani, M., Kishimoto, Y., Iizuka, M., Saita, E., Toyozaki, M., & Kondo, K. (2011). Sweet potato (*Ipomoea batatas* L.) leaves suppressed oxidation of low density lipoprotein (LDL) *in vitro* and in human subjects. *Journal of Clinical Biochemistry and Nutrition*, 48(3), 203-208.

Nair, S. S., Kavrekar, V., & Mishra, A. (2013). *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology*, 3(1), 128-132.

Nandutu, A. M., Clifford, M., & Howell, N. K. (2007). Analysis of phenolic compounds in Ugandan sweet potato varieties (NSP, SPK AND TZ). *African Journal of Biochemistry Research*, 1(3), 029-036.

Nedachi, T., & Kanzaki, M. (2006). Regulation of glucose transporters by insulin and extracellular glucose in C2C12 myotubes. *American Journal of Physiology-Endocrinology and Metabolism*, 291(4), E817-E828.

Niero, E. L., & Machado-Santelli, G. M. (2013). Cinnamic acid induces apoptotic cell death and cytoskeleton disruption in human melanoma cells. *Journal of Experimental & Clinical Cancer Research*, *32*(1), 31, doi: 10.1186/1756-9966-32-31.

Niki, E., Yoshida, Y., Saito, Y., & Noguchi, N. (2005). Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications*, 338(1), 668-676.

Niki, E. (2008). Lipid peroxidation products as oxidative stress biomarkers. *Biofactors*, 34(2), 171-180.

Niwa, A., Tajiri, T., & Higashino, H. (2011). *Ipomoea batatas* and *Agarics blazei* ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats. *Journal of Clinical Biochemistry and Nutrition*, *48*(3), 194-202.

Nnawuchi, P. C., Ubbaonu, C. N., Ezinwoke, S. O., & Eke Okoro, O. N (2002). On–farm Evaluation of the Acceptability of High Ratio (40% and 50%) sweet potato / wheat flour Compositability in Bakery Products, Buns, Chin-chin and Doughnut in Imo State In: Udeaolor A, T.O. Ezulike and

J. Aham (eds.) proceedings of the 16th Annual Zonal Research Extension, Farmer input Linkage system. (REFILs). Workshop southeast /south zone of Nigeria. 19th – 23rd November, 2001, 113-119.

Oates, P. J. (2002). Polyol pathway and diabetic peripheral neuropathy. *International Review of Neurobiology*, 50, 325-392.

Ogunrinola, O. O., Fajana, O. O., Olaitan, S. N., Adu, O. B., & Akinola, M. O. (2015). Anti-diabetic activity of *Ipomoea batatas* leaves extract: Effects on hepatic enzymes in alloxan-induced diabetic rats. *Research Journal of Medicinal Plant*, 9(5), 227-233.

Ojong, P. B., Njiti, V., Guo, Z., Gao, M., Besong, S., & Barnes, S. L. (2008). Variation of flavonoid content among sweet potato accessions. *Journal of the American Society for Horticultural Science*, 133(6), 819-824.

Okaka, J. C., Akobundu, E. N. T. & Okaka, A. N. C. (2006). Food and Human Nutrition an integrated approach, OCJ Academic Publishers, Enugu, 135-368.

Ola, M. S., Ahmed, M. M., Ahmad, R., Abuohashish, H. M., Al-Rejaie, S. S., & Alhomida, A. S. (2015). Neuroprotective effects of rutin in streptozotocin-induced diabetic rat retina. *Journal of Molecular Neuroscience*, 56(2), 440-448.

Olivier, D. K., van Wyk, B. E., & van Heerden, F. R. (2008). The chemotaxonomic and medicinal significance of phenolic acids in Arctopus and Alepidea (Apiaceae subfamily Saniculoideae). *Biochemical Systematics and Ecology*, 36(9), 724-729.

Omodamiro, R. M., Afuape, S. O., Njoku, C. J., Nwankwo, I. I. M., Echendu, T. N. C. & Edward, T. C. (2013). Acceptability and proximate composition of some sweet potato genotypes: Implication of

breeding for food security and industrial quality. *International Journal of Biotechnology and Food Science*, 1(5), 97-101.

Osime, E. O., Ediale, G. E., Omoti, C. E., & Famodu, A. A. (2008). Effect of sweet potato leaf (*Ipomoea batatas*) extract on some haematological parameters using rabbits. *Journal of Medicine and Biomedical Research*, 7(1-2).

Ozcan, T., Akpinar-Bayizit, A., Yilmaz-Ersan, L., & Delikanli, B. (2014). Phenolics in human health. *International Journal of chemical Engineering and applications*, 5(5), 393.

Padda, M. S., & Picha, D. H. (2007). Methodology optimization for quantification of total phenolics and individual phenolic acids in sweet potato (*Ipomoea batatas* L.) roots. *Journal of Food Science*, 72(7), C412-C416.

Padda, M. S. & Picha, D. H. (2008). Quantification of phenolic acids and antioxidant activity in sweet potato genotypes. *Scientia Horticulturae*, 119, 17-20.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., ... & Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American college of Nutrition*, 22(1), 18-35.

Padmaja, G., Sheriff, J. T., & Sajeev, M. S. (2012). Food Uses and Nutritional Benefits of Sweet Potato. *Fruit, Vegetable and Cereal Science and Biotechnology*, *6*(1), 115-123.

Padwal, R., Majumdar, S. R., Johnson, J. A., Varney, J., & McAlister, F. A. (2005). A systematic review of drug therapy to delay or prevent type 2 diabetes. *Diabetes Care*, 28(3), 736-744.

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., & Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry*, 143, 300-306.

Pal, S., Gautam, S., Mishra, A., Maurya, R., & Srivastava, A.K. (2015). Antihyperglycemic and antidyslipidemic potential of *Ipomoea batatas* leaves in validated diabetic animal models. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7, 176-186.

Panda, V., & Sonkamble, M. (2012). Anti-ulcer activity of *Ipomoea batatas* tubers (sweet potato). *Functional Foods in Health and Disease*, *2*(3), 48-61.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2(5), 270-278.

Park, S. Y., Lee, S. Y., Yang, J. W., Lee, J. S., Oh, S. D., Oh, S., & Cho, H. S. (2016). Comparative analysis of phytochemicals and polar metabolites from colored sweet potato (*Ipomoea batatas* L.) tubers. *Food Science and Biotechnology*, 25(1), 283-291.

Park, W. S., Bae, J. Y., Kim, H. J., Kim, S. H., Lee, H. S., Kwak, S. S., & Ahn, M. J. (2014). Antiulcer and antiadipogenic acitities of carotenoid and anthocyanin extracts from color-fleshed sweet potato. *Planta Medica*, 80(10), 20.

Park, C. E., Kim, M. J., Lee, J. H., Min, B. I., Bae, H., Choe, W., ... & Ha, J. (2007). Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. *Experimental & Molecular Medicine*, 39(2), 222-229.

Park, J. M., Lee, J. S., Song, J. E., Sim, Y. C., Ha, S. J., & Hong, E. K. (2015). Cytoprotective effect of hispidin against palmitate-induced lipotoxicity in C2C12 myotubes. *Molecules*, 20(4), 5456-5467.

Park, K., Gross, M., Lee, D. H., Holvoet, P., Himes, J. H., Shikany, J. M., & Jacobs, D. R. (2009). Oxidative stress and insulin resistance. *Diabetes Care*, 32(7), 1302-1307.

Pessin, J. E., & Saltiel, A. R. (2000). Signaling pathways in insulin action: molecular targets of insulin resistance. *Journal of Clinical Investigation*, 106(2), 165-169.

Pfundstein, B., El Desouky, S. K., Hull, W. E., Haubner, R., Erben, G., & Owen, R. W. (2010). Polyphenolic compounds in the fruits of Egyptian medicinal plants (Terminalia bellerica, Terminalia chebula and Terminalia horrida): characterization, quantitation and determination of antioxidant capacities. *Phytochemistry*, *71*(10), 1132-1148.

Poljsak, B., Šuput, D., & Milisav, I. (2013). Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 956792, 11 pages, 2013. doi:10.1155/2013/956792.

Pontiki, E., Hadjipavlou-Litina, D., Litinas, K., & Geromichalos, G. Novel cinnamic acid derivatives as antioxidant and anticancer agents: design, synthesis and modeling studies. *Molecules*. 2014, 19(7), 9655-9674.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, 48(8), 3396-3402.

Rains, J. L., & Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine*, 50(5), 567-575.

Rani, Y., & Sharma, N. K. (2003, February). NUTRACEUTICALS: AYURVEDA PERSPECTIVE. In *III WOCMAP Congress on Medicinal and Aromatic Plants-Volume 6: Traditional Medicine and Nutraceuticals*, 680, 131-136.

Ramachandran, B., Yu, G., & Gulick, T. (2008). Nuclear respiratory factor 1 controls myocyte enhancer factor 2A transcription to provide a mechanism for coordinate expression of respiratory chain subunits. *Journal of Biological Chemistry*, 283(18), 11935-11946.

Ramprasad, R., Madhusudhan, S., & Dhanapal, C. K. ISSN 0975-413X CODEN (USA): PCHHAX.

Randle, P. J. (1998). Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes/Metabolism Research and Reviews*, 14(4), 263-283.

Rautenbach, F., Faber, M., Laurie, S., & Laurie, R. (2010). Antioxidant capacity and antioxidant content in roots of 4 sweet potato varieties. *Journal of Food Science*, 75(5), C400-5. doi: 10.1111/j.1750-3841.2010.01631.x.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9), 1231-1237.

Rebaya, A., Belghith, S. I., Baghdikian, B., Leddet, V. M., Mabrouki, F., Evelyne, O., & Ayadi, M. T. (2015). Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae), 5 (1), 052-057.

Rengarajan, S. A. T. H. I. S. H., Rani, M., & Kumaresapillai, N. (2012). Study of ulcer protective effect of *Ipomea batatas* (L.) dietary tuberous roots (Sweet Potato). *Iranian Journal of Pharmacology Therapeutics*, *11*, 36-39.

Rizvi, S. I., & Mishra, N. (2009). Anti-oxidant effect of quercetin on type 2 diabetic erythrocytes. *Journal of Food Biochemistry*, 33(3), 404-415.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, 51(10), 2866-2887.

Rodrigues, N., da R., Barbosa Junior, J. L., & Barbosa, M. I. M. J. (2016). Determination of physico-chemical composition, nutritional facts and technological quality of organic orange and purple-fleshed sweet potatoes and its flours. *International Food Research Journal* 23(5): 2071-2078.

Rosa, G., Manco, M., & Vega, N. (2003). Insulin sensitivity inversely relates to skeletal muscle ACC2 mRNA in formerly obese subjects. *Obesity Research*, 11, 1306-1312.

Rose, I.M. & Vasanthakaalam, H. (2011). Comparison of the nutrient composition of four sweet potato varieties cultivated in Rwanda. *American Journal of Food and Nutrition*, 1(1), 34-38.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 588-590.

Russo, D., Valentão, P., Andrade, P. B., Fernandez, E. C., & Milella, L. (2015). Evaluation of antioxidant, antidiabetic and anticholinesterase activities of Smallanthus sonchifolius landraces and correlation with their phytochemical profiles. *International journal of molecular sciences*, 16(8), 17696-17718.

Sabu, M. C., & Kuttan, R. (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacology*, 81(2), 155-160.

Sabu, M. C., Smitha, K., & Kuttan, R. (2002). Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *Journal of Ethnopharmacology*, 83(1), 109-116.

Saidu, A. N., Oibiokpa, F. I., & Olukotun, I. O. (2014). Phytochemical screening and hypoglycemic effect of methanolic fruit pulp extract of *Cucumis sativus* in alloxan-induced diabetic rats. *Journal of Medicinal Plants Research*, 8, 1173-1178.

Salawu, S. O., Udi, E., Akindahunsi, A. A., Boligon, A. A., & Athayde, M. L. (2015). Antioxidant potential, phenolic profile and nutrient composition of flesh and peels from Nigerian white and purple skinned sweet potato (*Ipomea batatas* L.). *Asian Journal of Plant Science Research*, 5, 14-23.

Salunkhe and Kadam, (1998). Handbook of Vegetables. Science & Technology. CRC Press, New York, USA.

Sangeetha, K. S., Umamaheswari, S., Reddy, C. U. M., & Kalkura, S. N. (2016). Flavonoids: Therapeutic potential of natural pharmacological agents. *International Journal of Pharmaceutical Sciences and Research*, 7(10), 3924-3930

Sanoussi, A. F., Dansi, A., Ahissou, H., Adebowale, A., Sanni, L. O., Orobiyi, A., & Sanni, A. (2016). Possibilities of *sweet potato* [*Ipomoea batatas* (L.) Lam] value chain upgrading as revealed by physico-chemical composition of ten elites landraces of Benin. *African Journal of Biotechnology*, *15*(13), 481-489.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition*, 81(1), 215S-217S.

Schronk, M. J. (2010). The Sweet Potato: An Important Part of Human Health. *Department of Horticulture, Texas A and M University, College Station, TX 77843*.

Sears, B., & Perry, M. (2015). The role of fatty acids in insulin resistance. *Lipids in Health and Disease*, 14, 121, doi: 10.1186/s12944-015-0123-1.

Sebastián, D., Herrero, L., Serra, D., Asins, G., & Hegardt, F. G. (2007). CPT I overexpression protects L6E9 muscle cells from fatty acid-induced insulin resistance. *American Journal of Physiology-Endocrinology and Metabolism*, 292(3), E677-E686.

Sebaugh, J. L. (2011). Guidelines for accurate EC50/IC50 estimation. *Pharmaceutical Statistics,* 10(2), 128-134.

Senanayake, S. A., Ranaweera, K. K. D. S., Gunaratne, A., & Bamunuarachchi, A. (2013). Comparative analysis of nutritional quality of five different cultivars of sweet potatoes (Ipomea batatas (L) Lam) in Sri Lanka. *Food Science & Nutrition*, 1(4), 284-291.

Sekhar, R. V., McKay, S. V., Patel, S. G., Guthikonda, A. P., Reddy, V. T., Balasubramanyam, A., & Jahoor, F. (2011). Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. *Diabetes care*, 34(1), 162-167

Shekhar S, Mishra D, Buragohain, AK, Chakraborty S, Chakraborty, N. (2015). Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (Ipomoea batatas L.). *Food Chemistry*, 173: 957-965.

Shen, X. L., Wu, J. M., Chen, Y., & Zhao, G. (2010). Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food Hydrocolloids*, 24(4), 285-290.

Shih, C. C., Shlau, M. T., Lin, C. H., & Wu, J. B. (2014). *Momordica charantia* ameliorates insulin resistance and dyslipidemia with altered hepatic glucose production and fatty acid synthesis and AMPK phosphorylation in high-fat-fed mice. *Phytotherapy Research*, 28(3), 363-371.

Sicree, R, Shaw, J & Zimmet, P. (2006). The global burden. diabetes and impaired glucose tolerance. Prevalence and projections. In: Gan, D. ed. Diabetes Atlas, 3rd edition. Brussels: *International Diabetes Federation*, 16–103.

Silveira, L. R., Fiamoncini, J., Hirabara, S. M., Procópio, J., Cambiaghi, T. D., Pinheiro, C. H. J., & Curi, R. (2008). Updating the effects of fatty acids on skeletal muscle. *Journal of Cellular Physiology*, 217(1), 1-12.

Sinha, A. K. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47(2), 389-394.

Sinha, D., Satapathy, T., Dewangan, M. K., Kumar, A., & Roy, A. (2015). Recent status on carbohydrate metabolizing enzyme inhibitors in regulation of diabetes: a mechanism based review. *Journal of Applied Pharmaceutical Research*, 3(2), 1-7.

Srinivasan, S., & Pari, L. (2012). Ameliorative effect of diosmin, a citrus flavonoid against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. *Chemico-Biological Interactions*, 195(1), 43-51.

Steinberg, D. (2009). The LDL modification hypothesis of atherogenesis: an update. *Journal of lipid research*, *50*(Supplement), S376-S381.

Sugata, M., Lin, C. Y., & Shih, Y. C. (2015). Anti-inflammatory and anticancer activities of Taiwanese purple-fleshed sweet potatoes (*Ipomoea batatas* L. Lam) extracts. *BioMed Research International*, vol. 2015, Article ID 768093, 10 pages, doi:10.1155/2015/768093.

Sundarram, A., & Murthy, T. P. K. (2014). α-amylase production and applications: A review. *Journal of Applied & Environmental Microbiology*, 2(4), 166-175.

Suprayitno, E. (2015). Isolation and identification structure antioxidant active compounds of ethyl acetate fraction hypokotil *Bruguiera gymnorhiza* (L) Lamk. *International Journal of ChemTech Research*, 8, 1858-1867.

Surayia, Z., Mohammad, S., Allen, J., Butt, M. S., Umair, A., & Asif, J. (2008). Impact of sweet potato cultivars on blood glucose level in diabetic and healthy participants. *International Journal of Agriculture and Biology*, 10(3), 316-320.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), 63-68.

Szablewski, L. (2011). Glucose homeostasis–mechanism and defects. In Diabetes-Damages and Treatments. Book edited by Everlon Cid Rigobelo, ISBN 978-953-307-652-2.

Tabet, F., & Touyz, R. M. (2007). Reactive oxygen species, oxidative stress, and vascular biology in hypertension. *Comprehensive Hypertension*, Edition, 337-344.

Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology*, 52(2), 149-153.

Tan, S. L., Nakatani, M., & Komaki, K. (2007). Breeding of sweet potato In: Kang MS, Priyadarshan PM, editors. Breeding major food staples. *Blackwell publishing*, 333-364.

Tang, W., Li, S., Liu, Y., Huang, M. T., & Ho, C. T. (2013). Anti-diabetic activity of chemically profiled green tea and black tea extracts in a type 2 diabetes mice model via different mechanisms. *Journal of Functional Foods*, 5(4), 1784-1793.

Tang, Y., Cai, W., & Xu, B. (2015). Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China. *Food Science and Human Wellness*, 4(3), 123-132.

Tangvarasittichai, S. (2015). Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World Journal of Diabetes*, 6(3), 456-480.

Tappel, A. (2014). Selenium-glutathione peroxidase: properties and synthesis. *Current Topics in Cellular Regulation*, 24, 87-96.

Taylor, R. (2012). Insulin resistance and type 2 diabetes. *Diabetes*, 61(4), 778-779.

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B., & Vazifeshenas, M. R. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, 126(2), 180-185.

Teow, C. C., Truong, V. D., McFeeters, R. F., Thompson, R. L., Pecota, K. V., & Yencho, G. C. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food chemistry, 103(3), 829-838.

Tewe, O.O., Ojeniyi. F.E. & Abu O.A. (2003). Sweet potato Production, Utilization, and Marketing in Nigeria. Social Sciences Department, International Potato Center (CIP), Lima, Peru.

Tiganis, T. (2011). Reactive oxygen species and insulin resistance: the good, the bad and the ugly. *Trends in Pharmacological Sciences*, 32(2), 82-89.

Torres, L. L., Quaglio, N. B., de Souza, G. T., Garcia, R. T., Dati, L. M. M., Moreira, W. L., & Nitrini, R. (2011). Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. *Journal of Alzheimer's Disease*, 26(1), 59-68.

Tosetti, F., Noonan, D. M., & Albini, A. (2009). Metabolic regulation and redox activity as mechanisms for angioprevention by dietary phytochemicals. *International Journal of Cancer*, 125(9), 1997-2003.

Treutter, D. (1989). Chemical reaction detection of catechins and proanthocyanidins with 4-dimethylaminocinnamaldehyde. *Journal of Chromatography A*, 467, 185-193.

Tumwegamire, S., Kapinga, R., Zhang, D., Crissman, C., & Agili, S. (2004). Opportunities for promoting orange-fleshed sweet potato as a mechanism for combat vitamin-A deficiency in Sub-Saharan Africa. *African Crop Science Journal*, *12*(3), 241-252.

Turcotte, L. P., & Fisher, J. S. (2008). Skeletal muscle insulin resistance: roles of fatty acid metabolism and exercise. *Physical Therapy*, 88(11), 1279-1296.

United States Department of Agriculture (USDA), Agricultural Research Service, Nutrient Data Laboratory (2015) USDA National Nutrient Database for Standard Reference, Release 28. (retrieved 2016 March 11). Available from Internet: http://www.ars.usda.gov/ba/bhnrc/ndl.

Upadhyay, S., & Dixit, M. (2015). Role of polyphenols and other phytochemicals on molecular signaling. *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 504253, 15 pages, 2015. doi:10.1155/2015/504253.

van de Laar, F. A. (2008). Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. *Vascular Health and Risk Management*, 4(6), 1189-1195.

Van Gelder, W. M. J. (1981). Conversion factor from nitrogen to protein for potato tuber protein. Potato Research, 24(4), 423-425.

Veloso, R. V., Latorraca, M. Q., Arantes, V. C., Reis, M. A., Ferreira, F., Boschero, A. C., & Carneiro, E. M. (2008). Soybean diet improves insulin secretion through activation of cAMP/PKA pathway in rats. *The Journal of Nutritional Biochemistry*, 19(11), 778-784.

Verma, V. M. (2014). Sweet potato cultivation guide. Kosrae Agricultural Experiment Station, page 14.

Wakil, S. J., & Abu-Elheiga, L. A. (2009). Fatty acid metabolism: target for metabolic syndrome. *Journal of Lipid Research*, 50(Supplement), S138-S143.

Wang, L. S., & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, 269(2), 281-290.

Wang, Y. J., Zheng, Y. L., Lu, J., Chen, G. Q., Wang, X. H., Feng, J., & Sun, Q. J. (2010). Purple sweet potato color suppresses lipopolysaccharide-induced acute inflammatory response in mouse brain. *Neurochemistry International*, 56(3), 424-430.

Wang, H. Y., Kan, W. C., Cheng, T. J., Yu, S. H., Chang, L. H., & Chuu, J. J. (2014). Differential anti-diabetic effects and mechanism of action of charantin-rich extract of Taiwanese *Momordica charantia* between type 1 and type 2 diabetic mice. *Food and Chemical Toxicology*, 69, 347-356.

Wang, L., Yue, Z., Guo, M., Fang, L., Bai, L., Li, X., & Zhao, H. (2016). Dietary flavonoid hyperoside induces apoptosis of activated human LX-2 hepatic stellate cell by suppressing canonical NF-κB signaling. *BioMed Research International*, vol. 2016, Article ID 1068528, 10 pages, 2016. doi:10.1155/2016/1068528

Wang, Z. Q., Ribnicky, D., Zhang, X. H., Zuberi, A., Raskin, I., Yu, Y., & Cefalu, W. T. (2011). An extract of *Artemisia dracunculus* L. enhances insulin receptor signaling and modulates gene

expression in skeletal muscle in KK-A y mice. *The Journal of Nutritional Biochemistry*, 22(1), 71-78.

Wehash, F. E., Abpo-Ghanema, I. I., & Saleh, R. M. (2012). Some physiological effects of Momordica charantia and Trigonella foenum-graecum extracts in diabetic rats as compared with cidophage®. *Proceedings of World Academy of Science, Engineering and Technology*, 64, 1206-14.

Wen, H., Kang, J., Li, D., Wen, W., Yang, F., Hu, H., & Liu, C. (2016). Antifungal activities of anthocyanins from purple sweet potato in the presence of food preservatives. *Food Science and Biotechnology*, *25*(1), 165-171.

Woolfe, J. A. (1992). "Sweet potato: an untapped food resource", Cambridge Univ. Press and the International Potato Center (CIP). Cambridge, UK.

Wu, Q., Qu, H., Jia, J., Kuang, C., Wen, Y., Yan, H., & Gui, Z. (2015). Characterization, antioxidant and antitumor activities of polysaccharides from purple sweet potato. *Carbohydrate Polymers*, 132, 31-40.

Wu, H., Deng, X., Shi, Y., Su, Y., Wei, J., & Duan, H. (2016). PGC-1α, glucose metabolism and type 2 diabetes mellitus. *Journal of Endocrinology*, 229(3), R99-R115.

Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences*, 11(11), 1185-1200.

Xu, Y., Fan, M., Ran, J., Zhang, T., Sun, H., Dong, M., & Zheng, H. (2016). Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars. *Saudi Journal of Biological Sciences*, 23(3), 379-388.

Yamakawa, O. (2000). New cultivation and utilization system for sweet potato toward the 21st century. In: Potential of Root Crops for Food and Industrial Resources (edited by M. Nakatani & K. Komaki). Twelfth Symposium of International Society of Tropical Root Crops (ISTRC), 10–16 Sept., 2000, Tsukuba, Japan. pages 8-13.

Yang, H. Y., & Lee, T. H. (2015). Antioxidant enzymes as redox-based biomarkers: a brief review. *BMB Reports*, 48(4), 200-208.

Yap, A., Nishiumi, S., Yoshida, K. I., & Ashida, H. (2007). Rat L6 myotubes as an *in vitro* model system to study GLUT4-dependent glucose uptake stimulated by inositol derivatives. *Cytotechnology*, 55(2-3), 103-108.

Yilmazer-Musa, M., Griffith, A. M., Michels, A. J., Schneider, E., & Frei, B. (2012). Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. *Journal of Agricultural and Food Chemistry*, 60(36), 8924-8929.

Yin, Z., Zhang, W., Feng, F., Zhang, Y., & Kang, W. (2014). α-Glucosidase inhibitors isolated from medicinal plants. *Food Science and Human Wellness*, *3*(3), 136-174.

Yoshida, Y., Umeno, A., & Shichiri, M. (2013). Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity *in vivo*. *Journal of Clinical Biochemistry and Nutrition*, 52(1), 9-16.

Yoshimoto, M., Kido, M., Kurata, R., & Kobayashi, T. (2011). Antibacterial activity of sweet potato (*Ipomoea batatas* L.) fibre on food hygienic bacteria. *The Bulletin of the Institute of Minami-Kyushu Regional.* Science, Kagoshima Women's Junior College, 27: 5-17.

Zhang, L., Keung, W., Samokhvalov, V., Wang, W., & Lopaschuk, G. D. (2010). Role of fatty acid uptake and fatty acid β-oxidation in mediating insulin resistance in heart and skeletal muscle. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1801(1), 1-22.

Zhang, X., Chen, C., Li, Y., Chen, D., Dong, L., Na, W., Wu, C., Zhang, J. & Li, Y. (2016). Tadehaginosides A–J, Phenylpropanoid Glucosides from Tadehagi triquetrum, Enhance Glucose Uptake via the Upregulation of PPARγ and GLUT-4 in C2C12 Myotubes. *Journal of natural products*, 79(5), 1249-1258.

Zhao, R., Li, Q., Long, L., Li, J., Yang, R., & Gao, D. (2007). Antidiabetic activity of flavone from *Ipomoea batatas* leaf in non-insulin dependent diabetic rats. *International Journal of Food Science & Technology*, 42(1), 8.

ADDENDUM 1

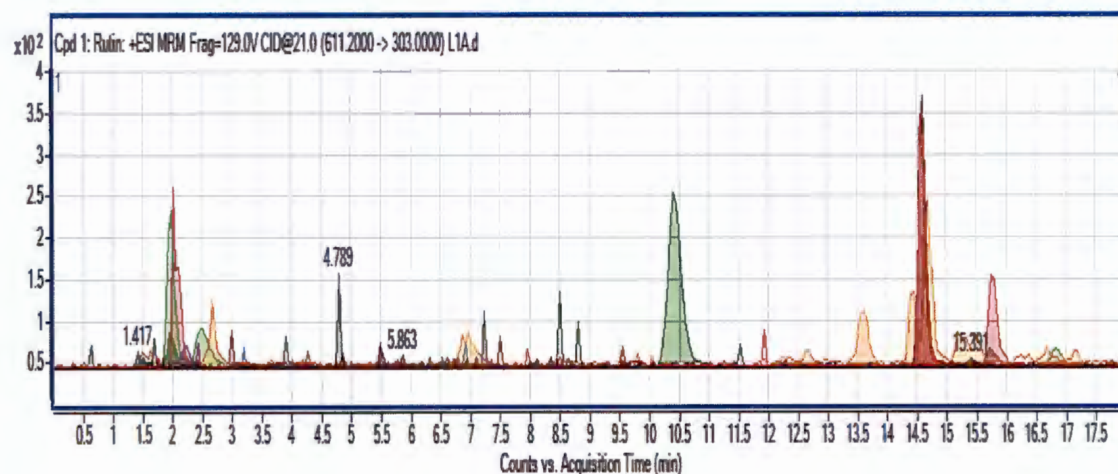
Tubers of selected South African grown cultivars of sweet potato. (a) Blesbok (b) Bophelo (c) Ndou (d) Monate (e) Mvuvhelo (f) Impilo (g) 199062.1

Source: Agriculture Research Centre (ARC) – Roodeplast, South Africa

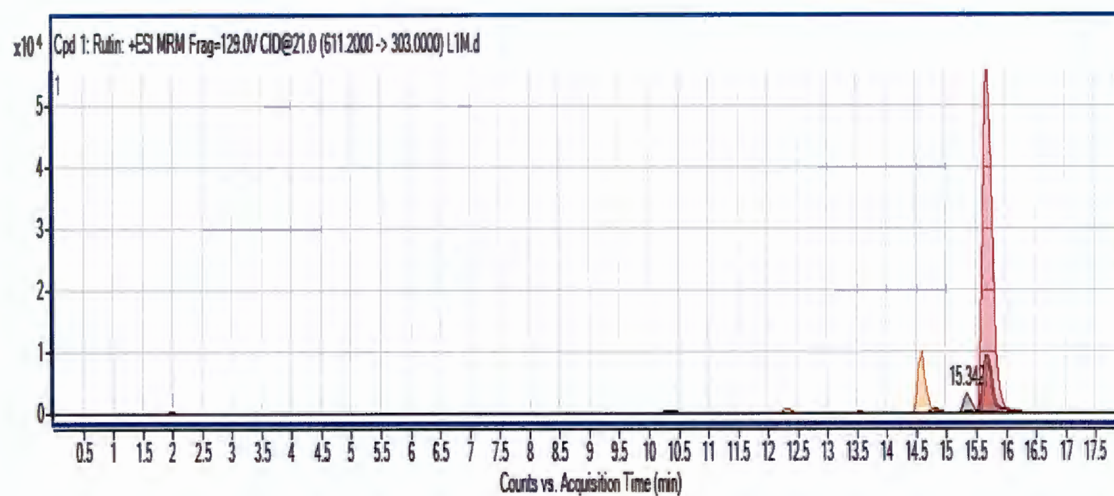
Leaves of selected South African grown cultivars of sweet potato. (a) Blesbok (b) Bophelo (c) Ndou (d) Monate (e) Mvuvhelo (f) Impilo (g) 199062.1

ADDENDUM 2

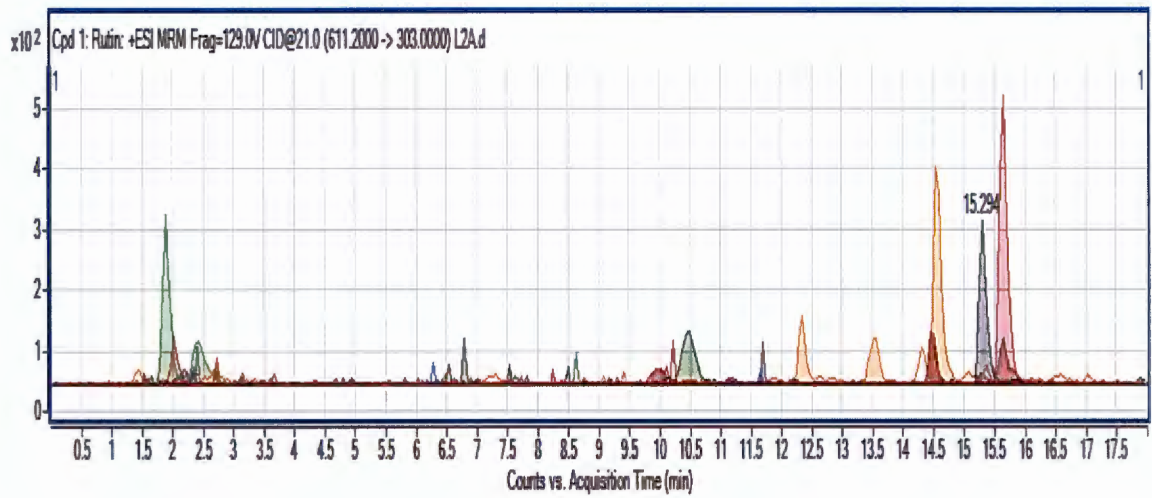
Individual chromatogram of the LC-MS analysis of the aqueous and aqueous-methanol extracts of the different cultivars of sweet potato grown in South Africa



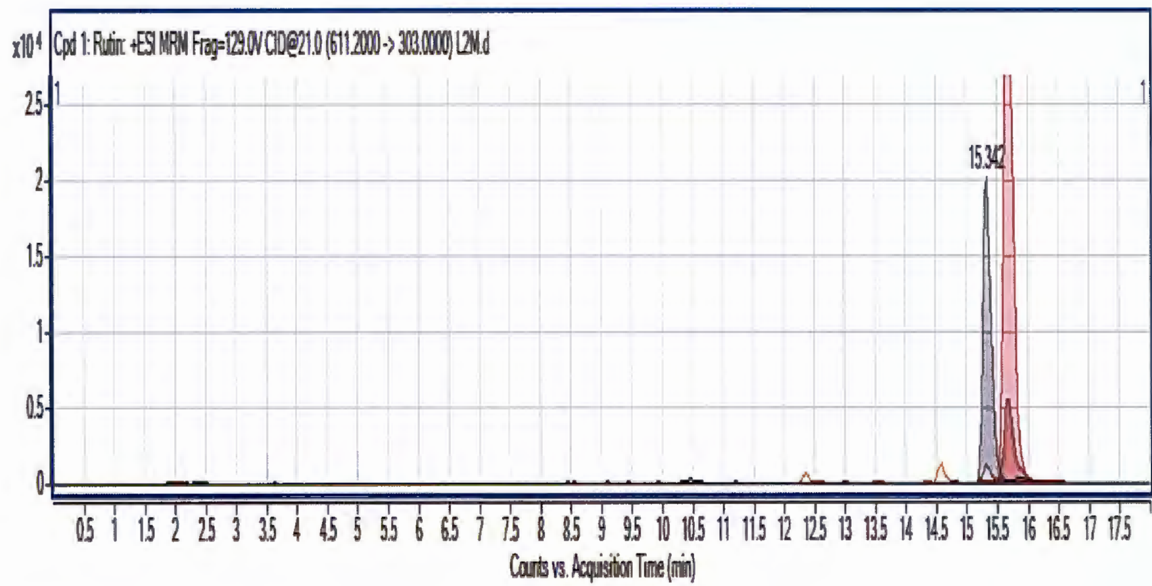
Aqueous extract of the leaves of Blesbok



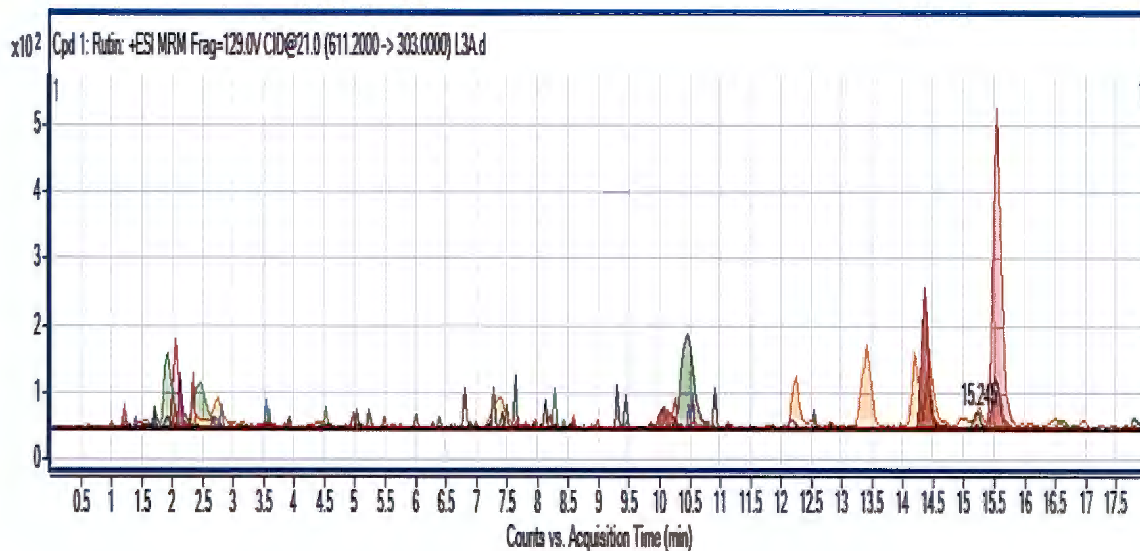
Aqueous-methanol extract of the leaves of Blesbok



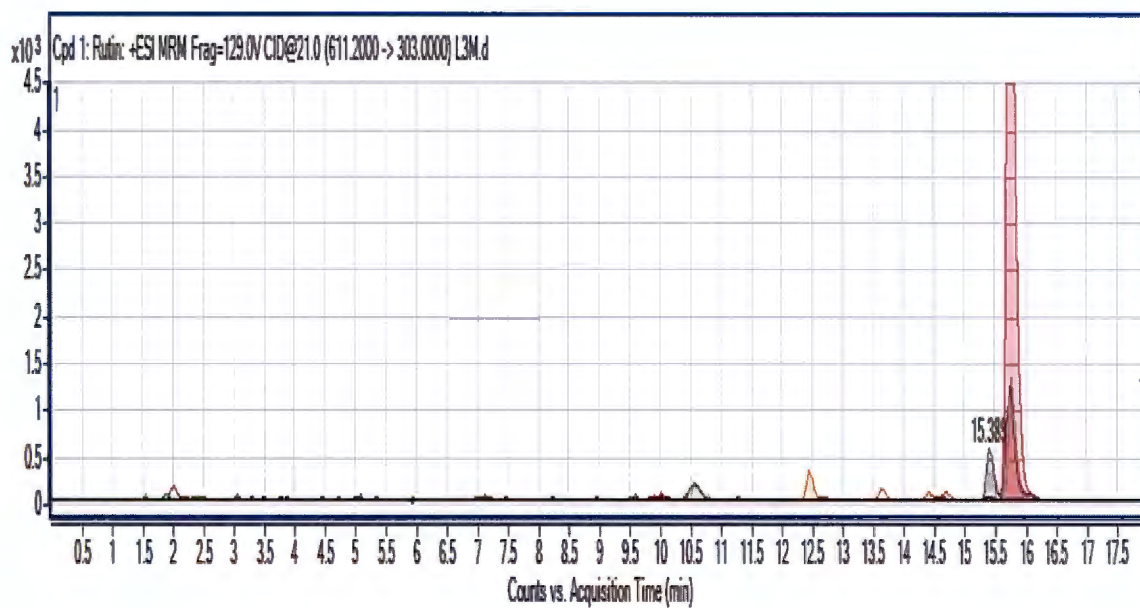
Aqueous extract of the leaves Bophelo



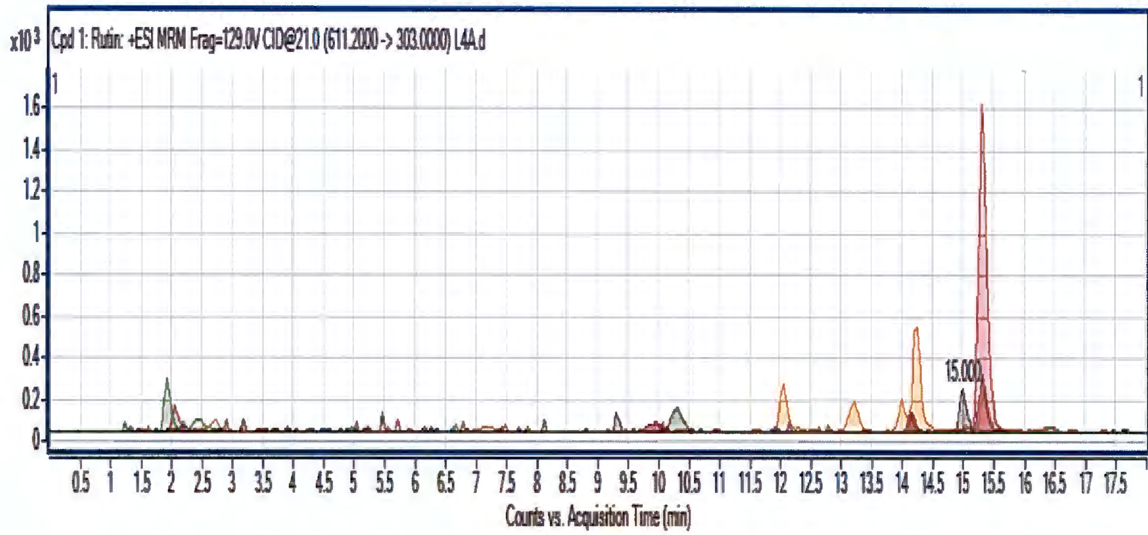
Aqueous-methanol extract of the leaves of Bophelo



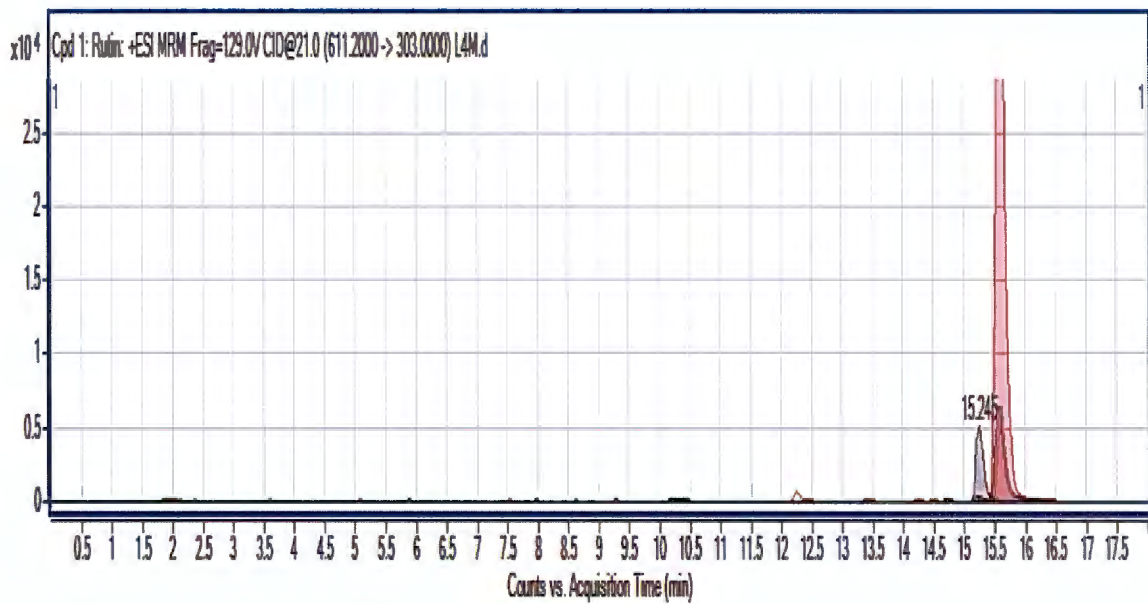
Aqueous extract of the leaves Ndou



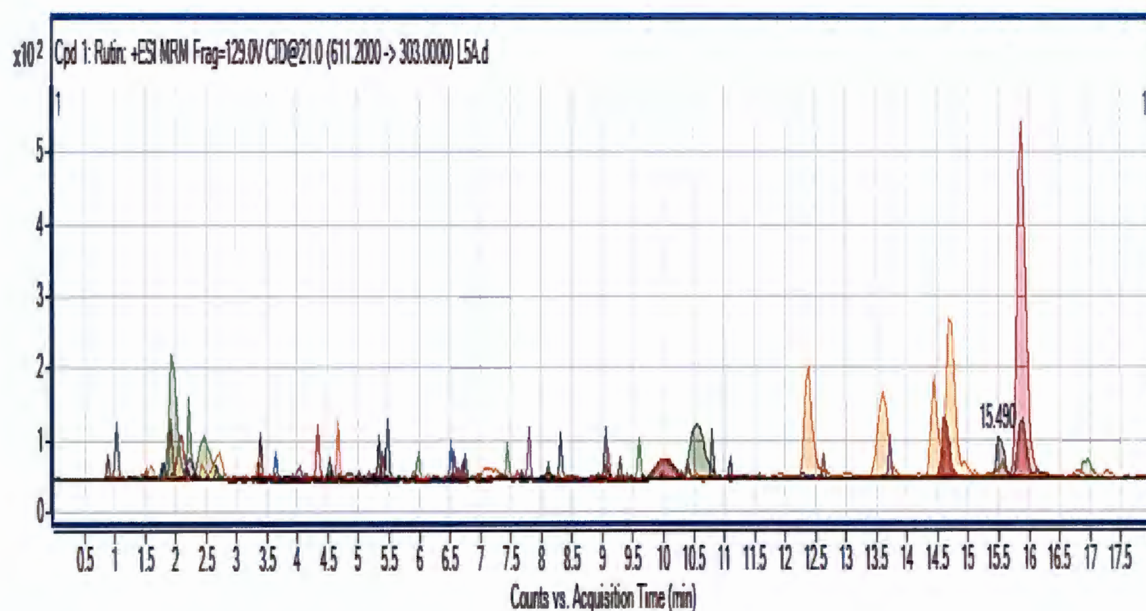
Aqueous-methanol extract of the leaves of Ndou



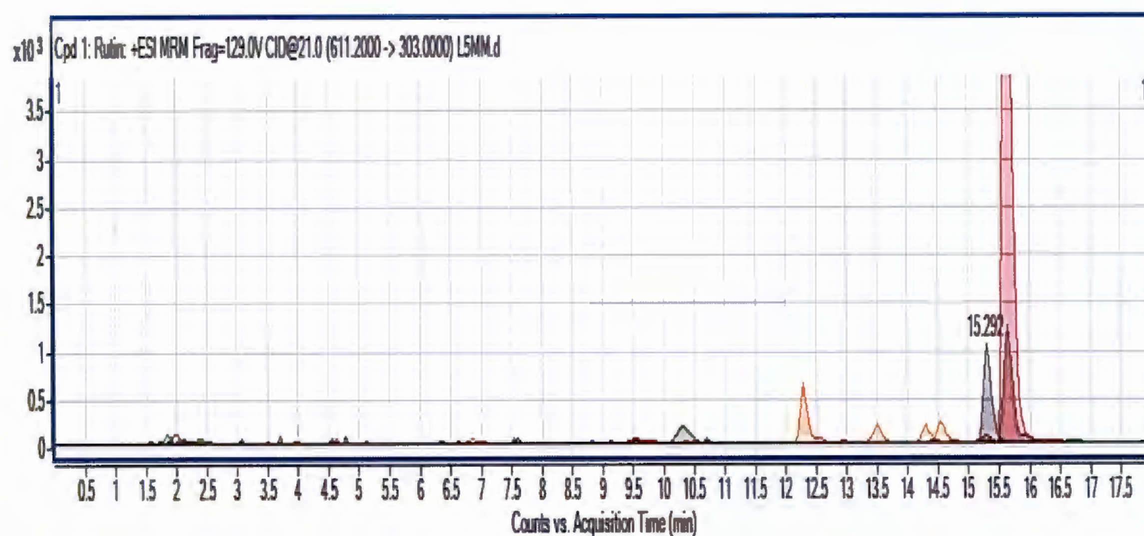
Aqueous extract of the leaves of Monate



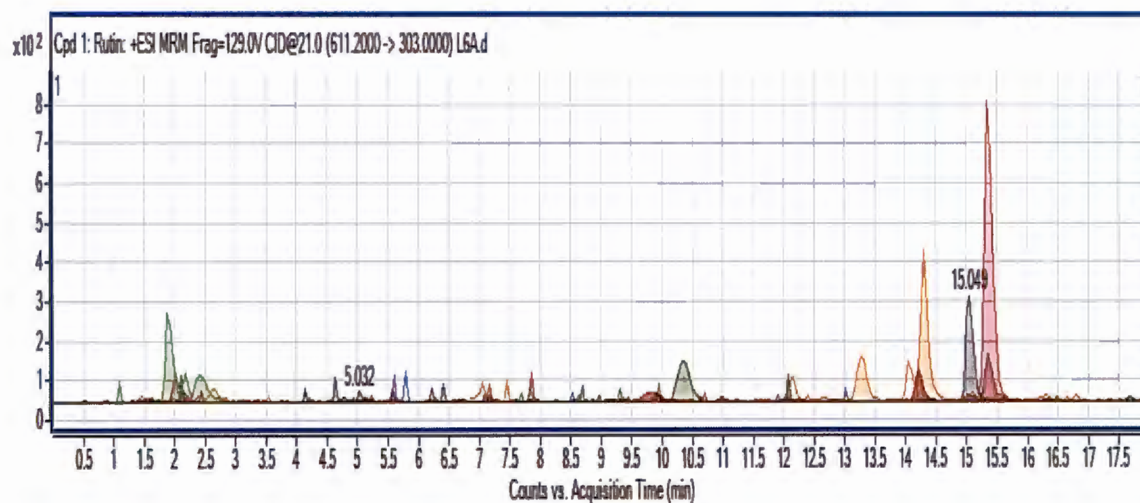
Aqueous extract of the leaves of Monate



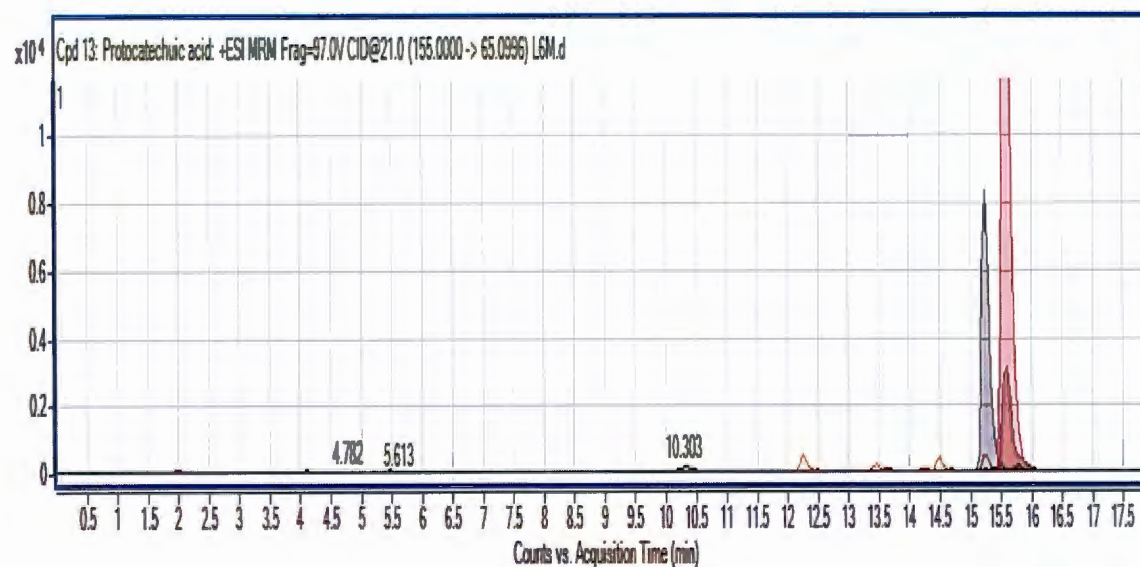
Aqueous extract of the leaves of Mvuvhelo



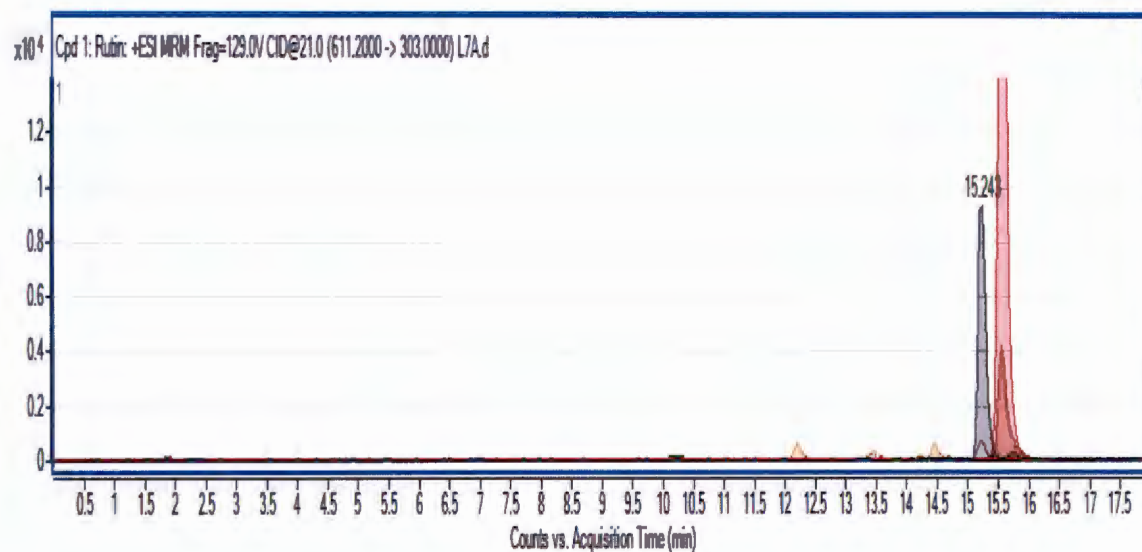
Aqueous-methanol extract of the leaves of Mvuvhelo



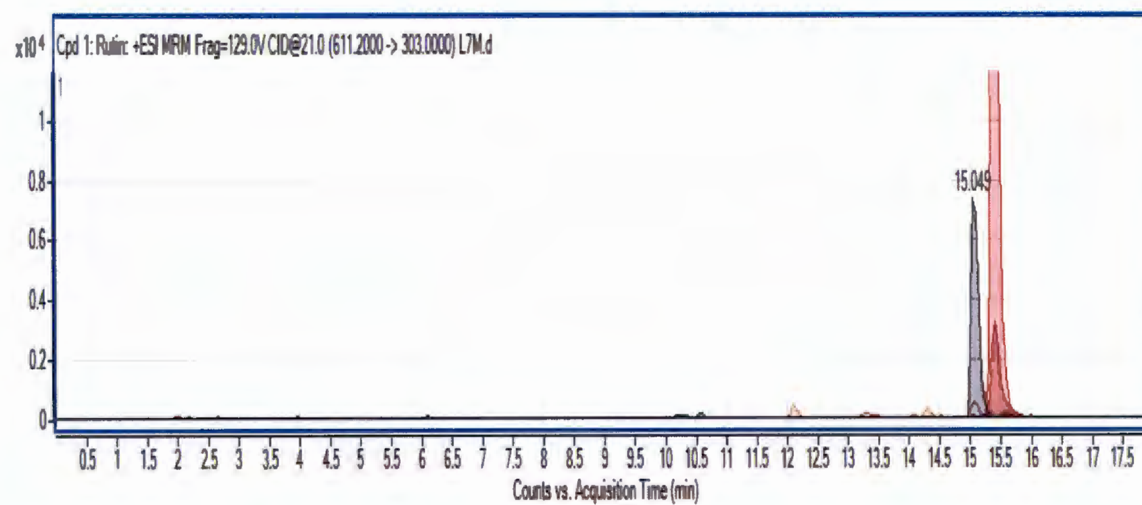
Aqueous extract of the leaves of Impilo



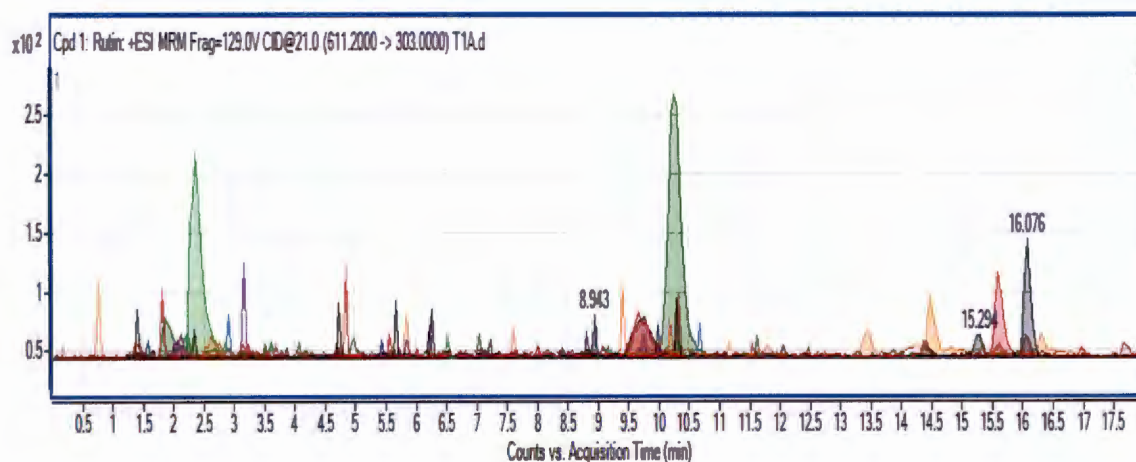
Aqueous-methanol extract of the leaves of Impilo



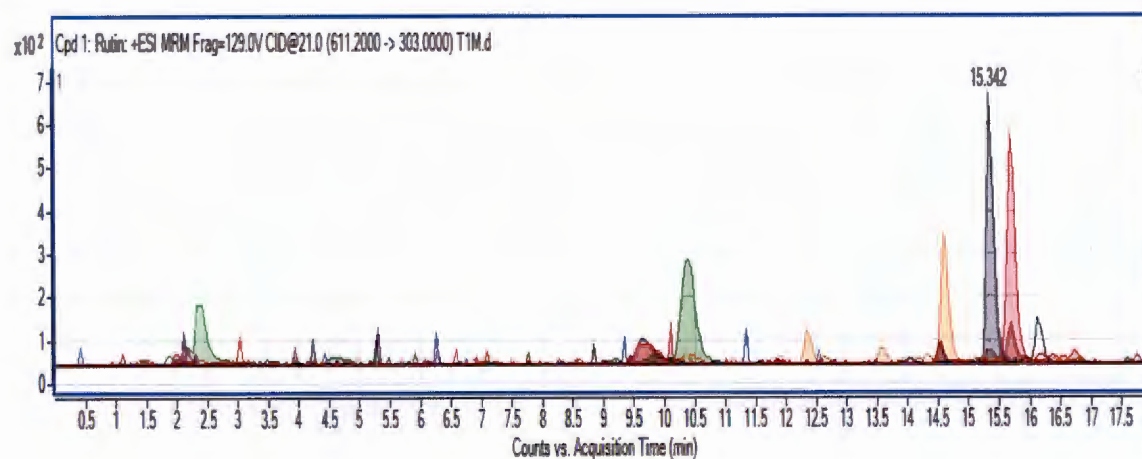
Aqueous extract of the leaves of 199062.1



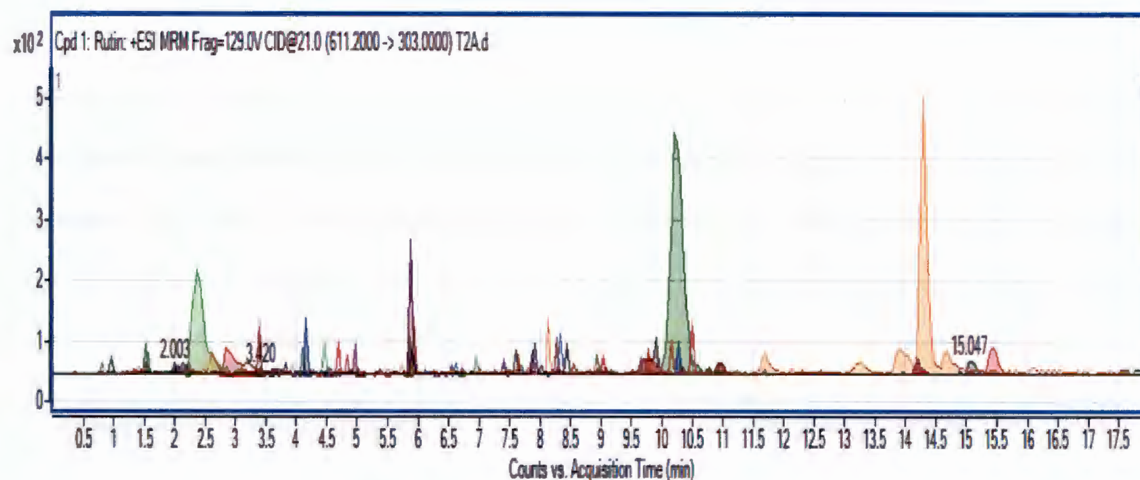
Aqueous-methanol extract of the leaves of 199062.1



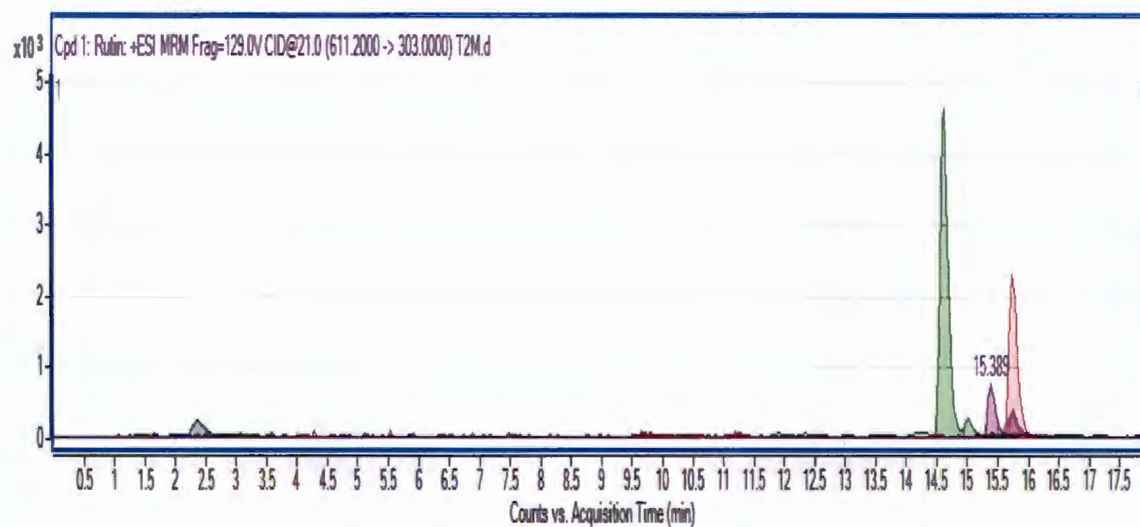
Aqueous extract of the tubers of Blesbok



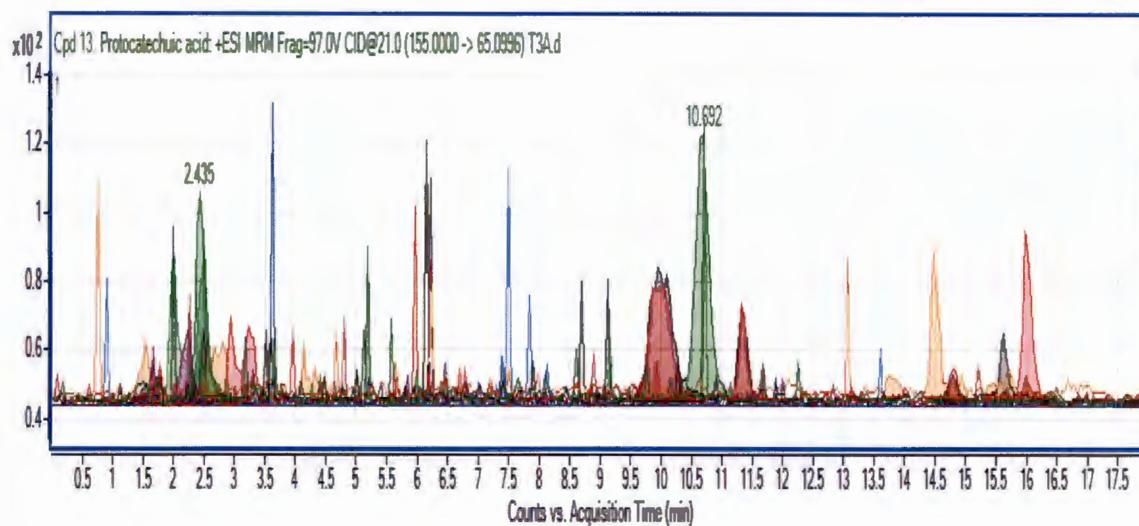
Aqueous-methanol extract of the tubers of Blesbok



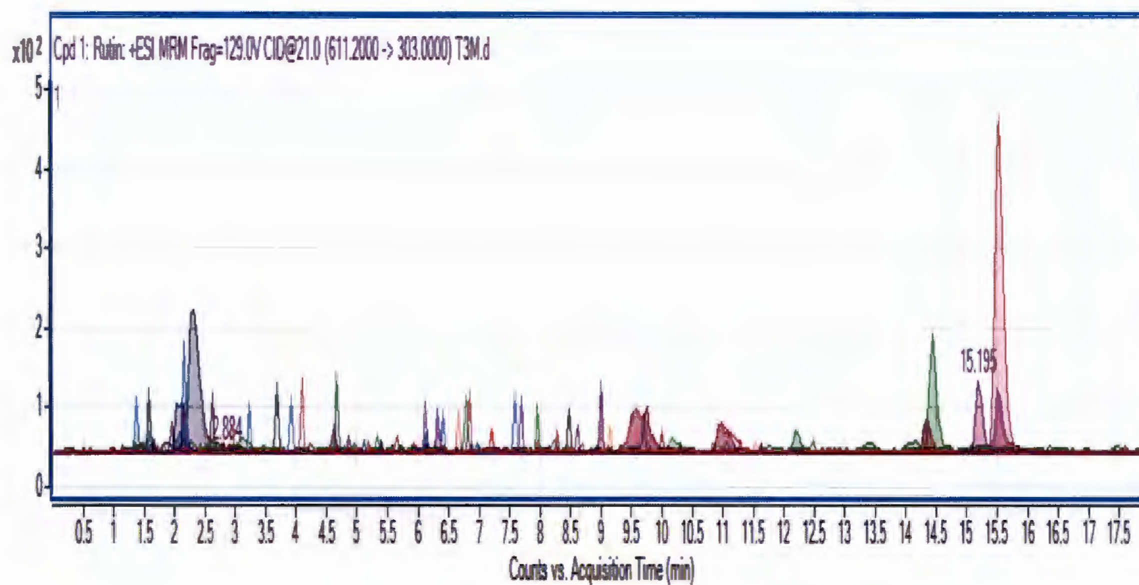
Aqueous extract of the tubers of Bophelo



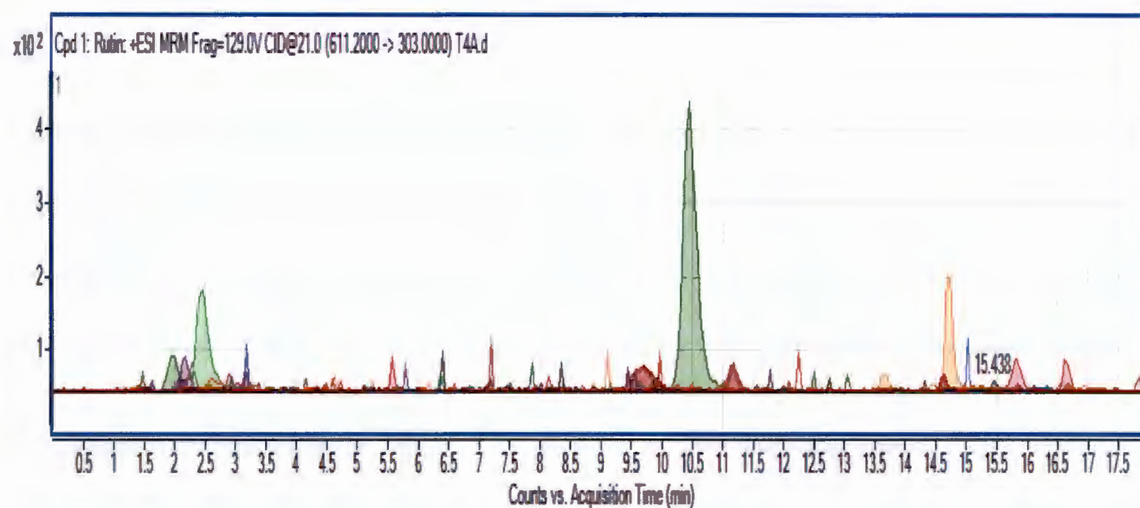
Aqueous-methanol extract of the tubers of Bophelo



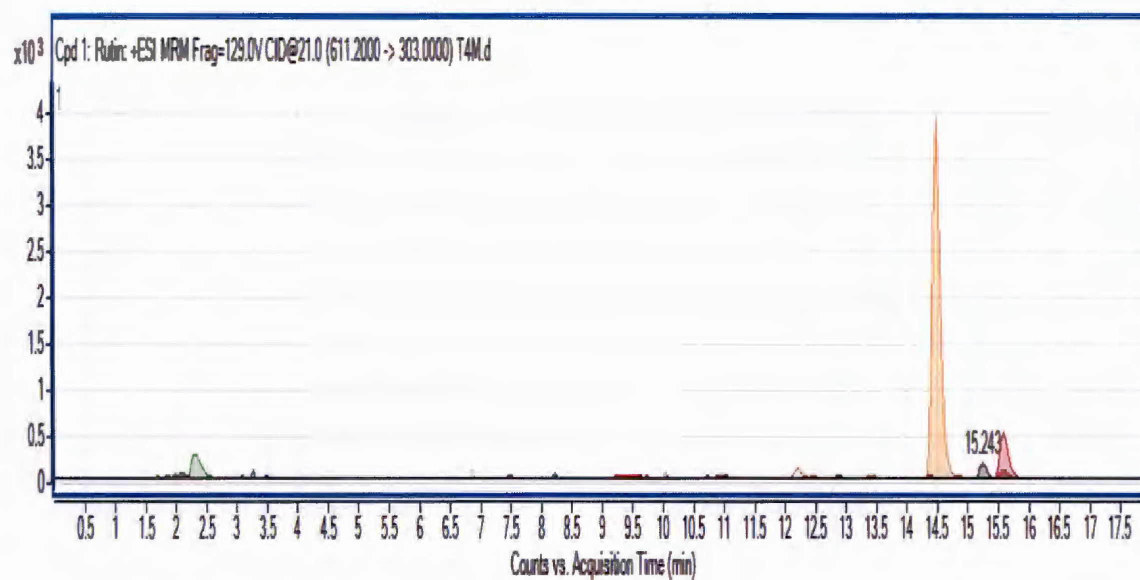
Aqueous extract of the tubers of Ndou



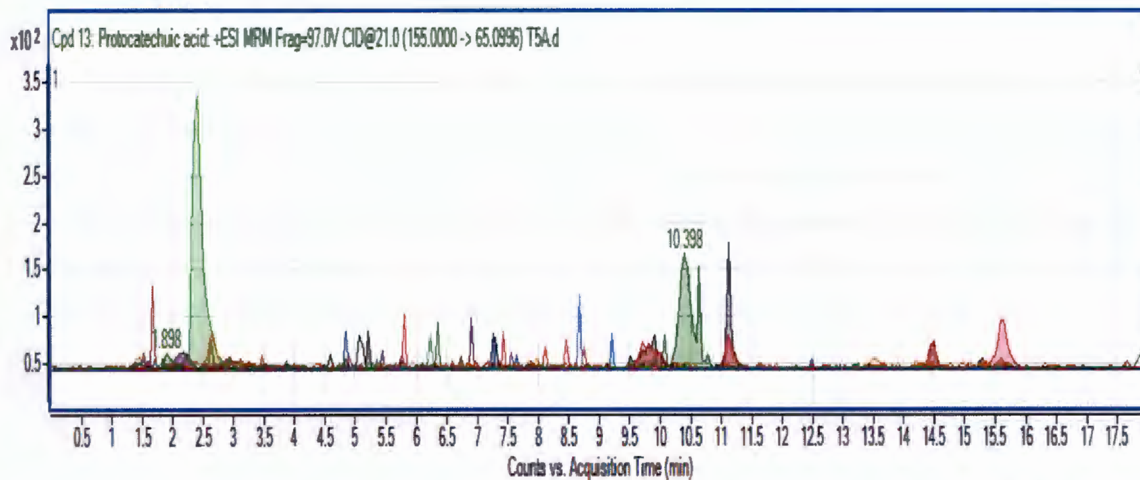
Aqueous-methanol extract of the tubers of Ndou



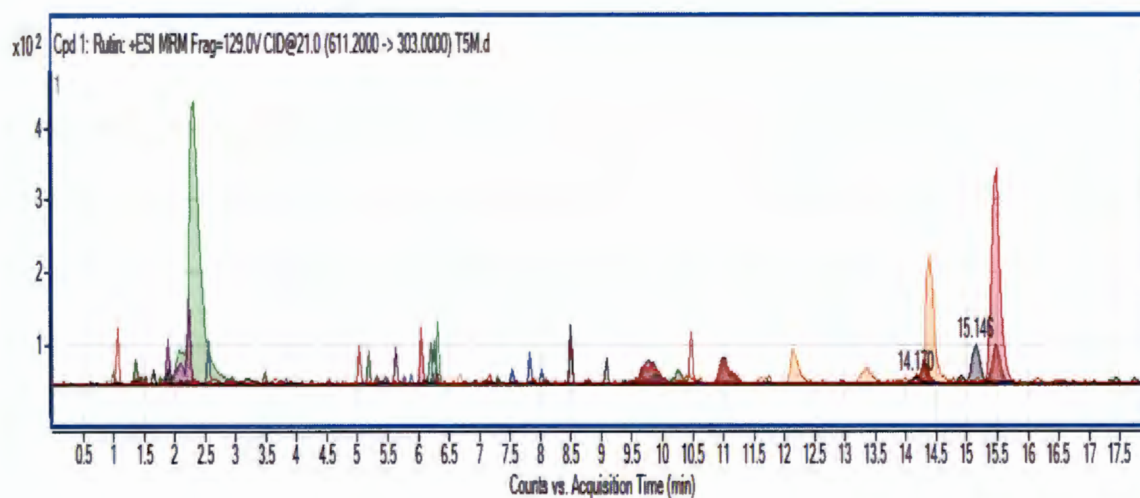
Aqueous extract of the tubers of Monate



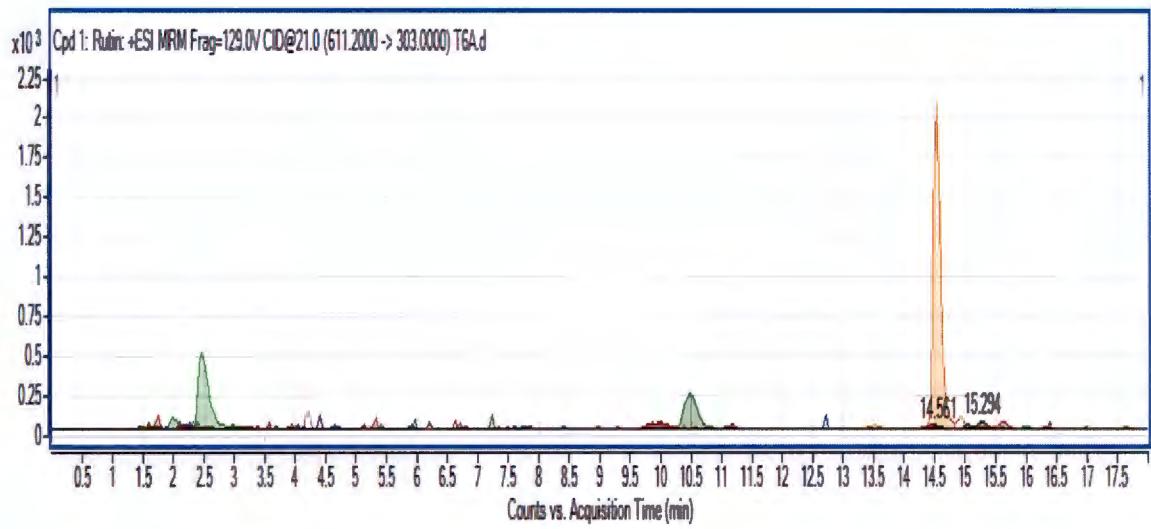
Aqueous-methanol extract of the tubers of Monate



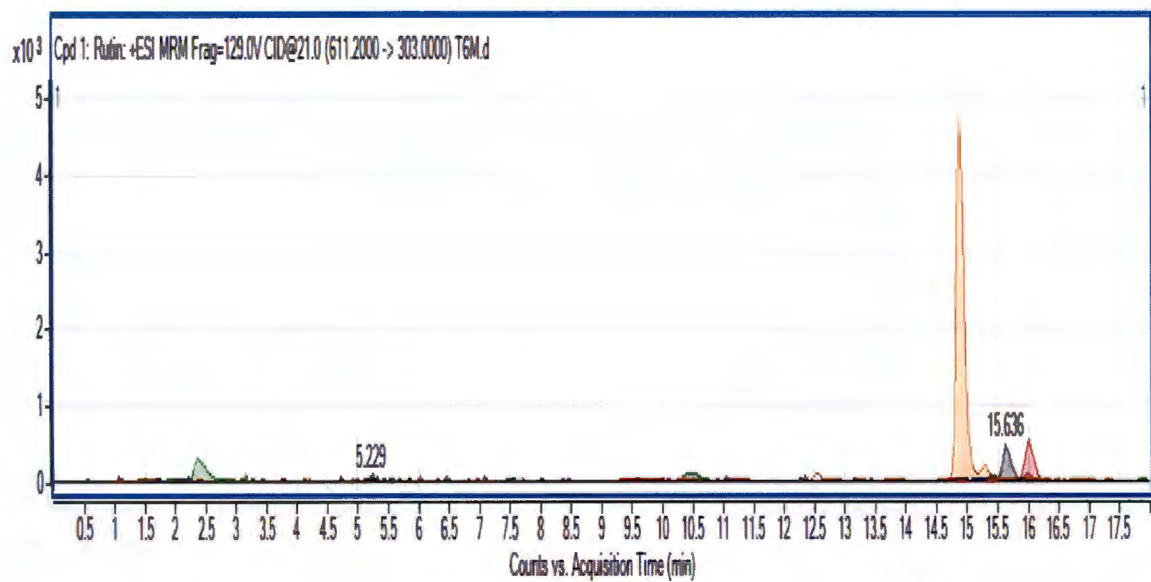
Aqueous extract of the tubers of Mvuvhelo



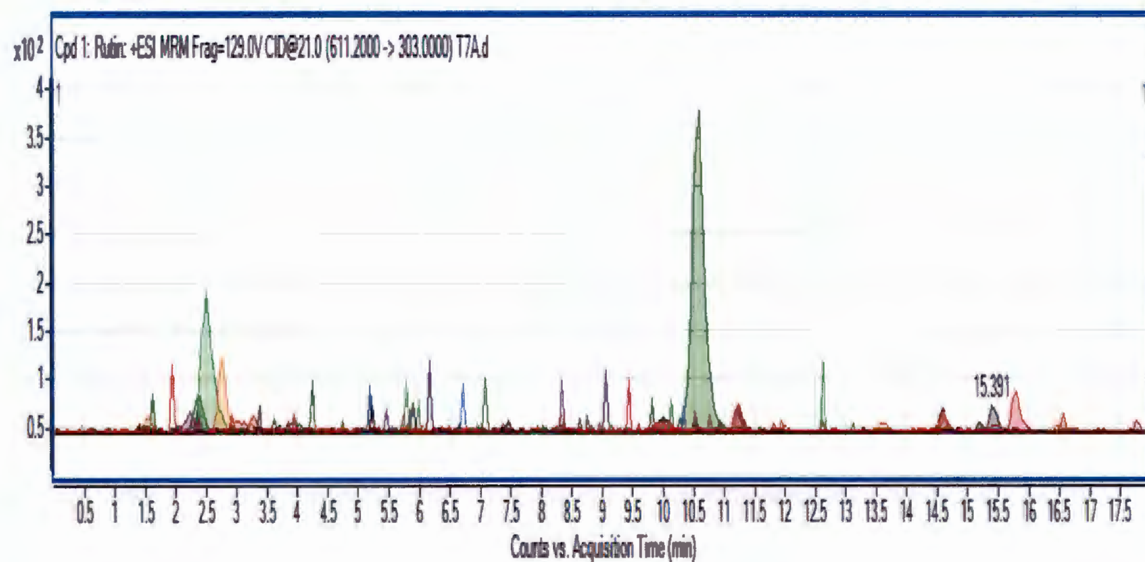
Aqueous-methanol extract of the tubers of Mvuvhelo



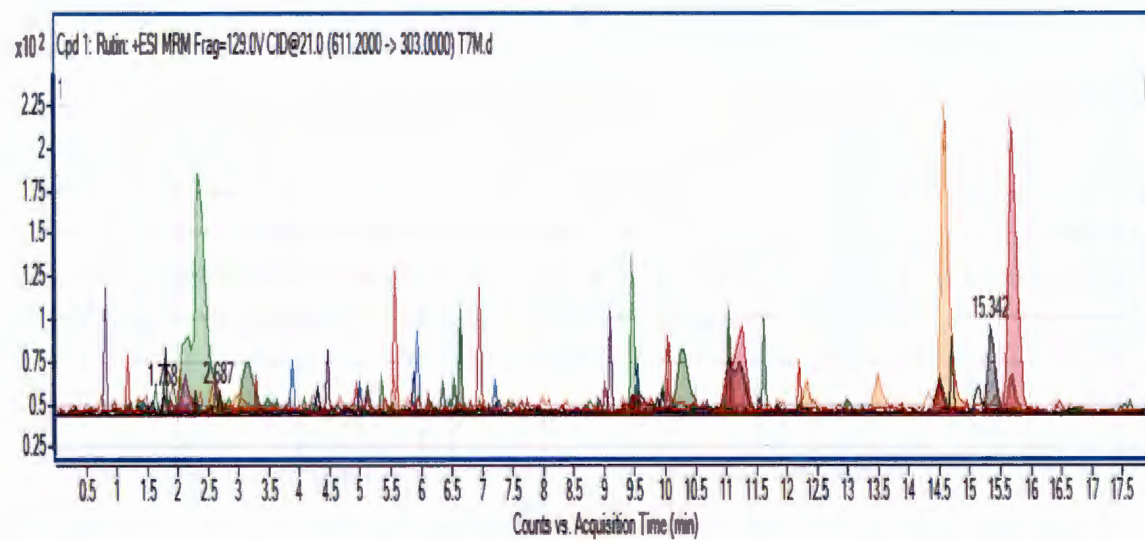
Aqueous extract of the tubers of Impilo



Aqueous-methanol extract of the tubers of Impilo



Aqueous extract of the tubers of 199062.1



Aqueous-methanol extract of the tuber of 199062.1